Attorney's Docket No.: MTS4USA

TRANSMITTAL LETTER TO THE U.S. ELECTED OFFICE (EO/US) - ENTRY INTO NATIONAL STAGE UNDER 35 USC 371

		March 11, 1999 April 1, 1999 July 21, 1999
PCT/CA00/00259	9 March 2000	
International Application No.	International Filing Date	Priority Date Claimed

SIALIC ACID-BINDING IG-LIKE LECTIN (SIGLEC) GENE; OB-BINDING PROTEIN LIKE (OB-BPL)
Title of Invention

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Applicant(s) for EO/US

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231
Attn: EO/US

Sir:

Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 USC 371:

(1) This express request to immediately begin national examination procedures (35 USC 371(f)).

(2) A copy of the cover sheet for the published International Application along with a copy of the specification as filed: 56 pages, including 3 pages of claims, 6 sheets of drawings, 7 pages of Sequence Listing and a copy of the 3 page International Search Report.

Express Mail No. ET033648209US

(3) a copy of the 4 page Request form.

(4) a first Preliminary Amendment for entry prior to calculation of the filing fees.

(5) our check in the amount of $860.00, covering the basic national fee as set forth in 37 CFR 1.492(a)(5) and based on the first Preliminary Amendment (16 total claims; 3 independent; and no multiple dependent).

(6) A Second Preliminary Amendment.

(7) Our check in the amount of $180.00, covering the extra claim fees after entry of the second Preliminary Amendment (30 total claims; 3 independent; and no multiple dependent).

Copies of the following miscellaneous items are also enclosed:

(8) Copy of the 4 page Demand for International Preliminary Examination.

(9) Copy of the 8 page Written Opinion.

(10) Copy of the 8 page International Preliminary Examination Report.

The executed Combined Declaration and Power of Attorney form will be filed by the appropriate deadline under 37 CFR §1.495(c)(2) with the surcharge under 37 CFR §1.492(e).

Please charge any additional fees which may be required to effect entry into the National Phase and credit any overpayment to Deposit Account No. 08-3040.

Please direct all communications concerning this application to the undersigned.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for the Applicants

By Mary E. Bak

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#9/C

MTS4USA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of) Group Art Unit:
)
George Foussias *et al*) Examiner:
)
Appln. No. 09/936,278)
)
International Appln Filing Date: March 9, 2000)
)
For: SIALIC ACID-BINDING IG-LIKE)
LECTIN (SIGLEC) GENE; OB BINDING)
PROTEIN LIKE (OB-BPL)) April 29, 2002

Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT C

Sir:

Kindly enter this preliminary amendment.

IN THE SPECIFICATION

On pages 26-27, replace the paragraph starting at page 26, line 32 and extending through page 27, line 2 with the following:

--Examination of the transmembrane and intracellular domains of Siglec family members reveals that these domains are more variable than the extracellular domain. However, there are regions that show a high level of conservation. As shown in Figure 4, all the Siglecs possess a single transmembrane domain, consisting of approximately 25 residues. In addition, within the cytoplasmic domain, there are two highly conserved motifs. The first of these, L(HQ)YA(SV)L (SEQ ID NO: 14), exhibits similarity to an immunoreceptor tyrosine kinase inhibitory motif (ITIM) (SEQ ID NO: 15), which has a 6 amino acid consensus sequence (ILV)xYxx(LV) (SEQ ID NO: 16) (Burshtyn *et al.,* 1997; Vivier and Daeron 1997). The second

motif, TEYSE(IV) (SEQ ID NO: 17), is homologous to a sequence (TxYxx(IV)) (SEQ ID NO: 18) recently found in the signaling lymphocyte activation molecule (SLAM) which is responsible for the binding of the SLAM-associated protein (SAP) (Coffey *et al.,* 1998; Sayos *et al.,* 1998).--

On page 36, replace the entirety of Table 5 and the notes with the following:

Table 5. Predicted exons of the unknown gene UG. The translated protein sequences of each exon (open reading frame) are shown.

Exon No.	Putative coding region[1]		No. of bases	Translated protein sequence	EST match[2]	Intron phase [3]	Stop codon[4]	Exon prediction program [5]	SEQ ID NO.
	From (bp)	To (bp)							
1	44,129	44,641	513	PPLSLEPAVPERRTLRNRRSLAALAPLTPDMLLLLL PLLWGRERAEGQTSKLLTMQSSVTVQEGLCVHVP CSFSYPSHGWIYPGPVVHGYWFREGANTDQDAPV ATNNPARAVWEETRDRFHLLGDPHTKNCTLSIRD ARRSGAGRYFFRMEKGSIKWNYKHHRLSVNVT	+	I	-	B,C	AA1-170 of SEQ ID NO: 2
2	44,843	45,121	279	ALTHRPNILIPGTLESGCPQNLTCSVPWACEQGTPP MISWIGTSVSPLDPSTTRSSVLTLIPQPQDHGTSLTC QVTFPGASVTTNKTVHLNVS	+	I	-	A, B, C, D	AA171-263 of SEQ ID NO: 2
3	45,327	45,374	48	YPPQNLTMTVFQGDGT	-	I	-	A,B,D	AA264-278 of SEQ ID NO: 2
4	46,318	46,542	225	EGQSLRLVCAVDAVDSNPPARLSLSWRGLTLCPSQ PSNPGVLELPWVHLRDAAEFTCRAQNPLGSQQVY LNVSLQ	+	I	-	A,B,C	AA262-336 of SEQ ID NO: 3
5	47,195	47,283	186	SKATSGVTQGVVGGAGATALVFLSFCVIFV	+	0	-	A, B, C, D	AA337-366 of SEQ ID NO: 3
6	49,136	49,554	186	GPLTEPWAEDSPPDQPPPASARSSVGEGELQYASL SFQMVKPWDSRGQEATDTEYSEIKIHR	+	-	+	A, B, C	AA400-461 of SEQ ID NO: 3

* All footnotes same as Table 2.

1. conventional numbering of exons in comparison to the five coding exons of PSA. Nucleotide numbers refer to the related contig (see text).
2. (+) = >95% homology with published human EST sequences.
3. Intron phase:0=the intron occurs between codons; I=the intron occurs after the first nucleotide of the codon; II=the intron occurs after the second nucleotide of the codon.
4. (+) denotes the exon containing the stop codon.
5. H=histidine, D=aspartic acid, S=serine. The amino acids of the catalytic triad are bold and underlined. A=GeneBuilder (exon analysis), C=Grail 2, D=GENEID-3.

REMARKS

The above amendments to the specification were made to correct obvious typographical and grammatical errors, and to insert missing sequence identification numbers. No new matter is introduced with these amendments. Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

Further submitted with this amendment is a substitute Sequence Listing in computer readable form as required by 37 CFR §1.821(e) and a substitute paper copy of the Sequence Listing as required by 37 CFR §1.821(c), as well as the following Statement required under those rules.

The Sequence Listing has been amended to conform it with the PatentIn Version 3.1 and properly identify the inventors and priority data.

Applicants request that these amendments and substitute sequence listing materials be entered into the specification, and that the application proceed to issuance in due course.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for the Applicants

By Mary E. Bak

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Spring House, PA 19477
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Telefacsimile: (215) 540-5818

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

On pages 26-27, replace the paragraph starting at page 26, line 32 and extending through page 27, line 2 with the following:

--Examination of the transmembrane and intracellular domains of Siglec family members reveals that ~~it is~~ these domains are more variable than the extracellular domain. However, there are regions that show a high level of conservation. As shown in Figure 4, all the Siglecs possess a single transmembrane domain, consisting of approximately 25 residues. In addition, within the cytoplasmic domain, there are two highly conserved motifs. The first of these, L(HQ)YA(SV)L (SEQ ID NO: 14), exhibits similarity to an immunoreceptor tyrosine kinase inhibitory motif (ITIM) (SEQ ID NO: 15), which has a 6 amino acid consensus sequence (ILV)xYxx(LV) (SEQ ID NO: 16) (Burshtyn *et al.,* 1997; Vivier and Daeron 1997). The second motif, TEYSE(IV) (SEQ ID NO: 17), is homologous to a sequence (TxYxx(IV)) (SEQ ID NO: 18) recently found in the signaling lymphocyte activation molecule (SLAM) which is responsible for the binding of the SLAM-associated protein (SAP) (Coffey *et al.,* 1998; Sayos *et al.,* 1998).--

On page 36, replace the entirety of Table 5 and the notes with the following:

Table 5. Predicted exons of the unknown gene UG. The translated protein sequences of each exon (open reading frame) are shown.

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2	44,843	45,121	279	ALTHRPNILIPGTLESGCPQNLTCSVPWACEQGTPP MISWIGTSVSPLDPSTTRSSVLTLIPQPQDHGTSLTC QVTFPGASVTTNKTVHLNVS	+	I	-	A, B, C, D	AA171-263 of SEQ ID NO: 2
3	45,327	45,374	48	YPPQNLTMTVFQGDGT	-	I	-	A,B,D	AA264-278 of SEQ ID NO: 2
4	46,318	46,542	225	EGQSLRLVCAVDAVDSNPPARLSLSWRGLTLCPSQ PSNPGVLELPWVHLRDAAEFTCRAQNPLGSQQVY LNVSLQ	+	I	-	A,B,C	AA262-336 of SEQ ID NO: 3
5	47,195	47,283	186	SKATSGVTQGVVGGAGATALVFLSFCVIFV	+	0	-	A, B, C, D	AA337-366 of SEQ ID NO: 3
6	49,136	49,554	186	GPLTEPWAEDSPPDQPPPASARSSVGEGELQYASL SFQMVKPWDSRGQEATDTEYSEIKIHR	+	-	+	A, B, C	AA400-461 of SEQ ID NO: 3

* All footnotes same as ~~table~~ Table 2.

1. conventional numbering of exons in comparison to the five coding exons of PSA. Nucleotide numbers refer to the related contig (see text).
2. (+) = >95% homology with published human EST sequences.
3. Intron phase:0=the intron occurs between codons; I=the intron occurs after the first nucleotide of the codon; II=the intron occurs after the second nucleotide of the codon.
4. (+) denotes the exon containing the stop codon.
5. H=histidine, D=aspartic acid, S=serine. The amino acids of the catalytic triad are bold and underlined. A=GeneBuilder (exon analysis), C=Grail 2, D=GENEID-3.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	) Group Art Unit:
George Foussias et al	) Examiner:
Appln. No.	)
Filed: Herewith	)
For: SIALIC ACID-BINDING IG-LIKE LECTIN (SIGLEC) GENE; OB-BINDING PROTEIN LIKE (OB-BPL)	) September 10, 2001

Assistant Commissioner for Patents
Box PCT
Washington, DC 20231

PRELIMINARY AMENDMENT B

Sir:

Please amend the above-identified patent application as follows.

In the Specification

Page 1, line 2, before "Field of the Invention", insert the following new paragraph:

-- Cross-Reference to Related Applications

This is a 371 of PCT/CA00/00259, filed March 9, 2000, which claims the benefit of the priorities of U. S. Patent Application No. 60/144,919, filed July 21, 1999, now abandoned, U. S. Patent Application No. 60/127,386, filed April 1, 1999, now abandoned, and U. S. Patent Application No. 60/124,260, filed March 11, 1999, now abandoned. --

Express Mail No. ET033648209US

Please enter the attached Abstract of the Disclosure on the attached page as new page 40.

<u>In the Claims</u>

Amend claims 2-3, 8, 11-14, 16-18, 20, and 25-26 as follows.

2. (Amended) The isolated nucleic acid molecule according to claim 1 which comprises:

(i) a nucleic acid sequence encoding a polypeptide having substantial sequence identify with the amino acid sequence shown in Table 5 or SEQ ID NO: 2 or SEQ ID NO: 3;

(ii) nucleic acid sequences complementary to (i);

(iii) a degenerate form of a nucleic acid sequence of (i);

(iv) a nucleic acid sequence comprising at least 18 nucleotides and capable of hybridizing to a nucleic acid sequence in (i), (ii), or (iii);

(v) a nucleic acid sequence encoding a truncation, an analog, an allelic or species variation of a polypeptide comprising the amino acid sequence shown in Table 5 or SEQ ID NO: 2, or SEQ ID NO: 3; or

(vi) a fragment, or allelic or species variation of (i), (ii) or (iii).

3. (Amended) The isolated nucleic acid molecule according to claim 1 which comprises:

(a) a nucleic acid sequence having substantial sequence identity or sequence similarity with a nucleic acid sequence of SEQ ID NO: 1;

(b) nucleic acid sequences complementary to (i), preferably complementary to the full nucleic acid sequence of SEQ ID NO: 1;

(c) nucleic acid sequences differing from any of the nucleic acid sequences of (i) or (ii) in codon sequences due to the degeneracy of the genetic code; or

(d) a fragment, or allelic or species variation of (i), (ii) or (iii).

8. (Amended) An isolated protein selected from the group consisting of a protein comprising an amino acid sequence of SEQ. ID. NO. 2 or 3 and a protein having at least 65% amino acid sequence identity to an amino acid sequence of SEQ. ID. NO. 2 or 3.

11. (Amended) A protein prepared in accordance with the method of claim 30.

12. (Amended) An antibody having specificity against an epitope of a polypeptide of claim 8.

13. (Amended) An antibody according to claim 12 labeled with a detectable substance and used to detect the polypeptide in biological samples, tissues, and cells.

14. (Amended) A probe comprising a sequence encoding a protein according to claim 8, or a part thereof.

16. (Amended) A method according to claim 31 wherein the condition is cancer or a disorder of the hematopoietic system.

17. (Amended) A method for identifying a substance which associates with a protein according to claim 8 comprising (a) reacting the protein with at least one substance which potentially can associate with the protein, under conditions which permit the association between the substance and protein, and (b) removing or detecting protein associated with the substance, wherein detection of associated protein and substance indicates the substance associates with the protein.

18. (Amended) A method for evaluating a compound for its ability to modulate the biological activity of a protein according to claim 8 comprising providing a known concentration of the protein with a substance which associates with the protein and a test compound under conditions which permit the formation of complexes between the substance and protein, and removing and/or detecting complexes.

20. (Amended) A method according to claim 19 wherein nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

25. (Amended) A transgenic non-human mammal which does not express an OB-BPL protein according to claim 8 resulting in an OB-BPL associated pathology.

26. (Amended) A transgenic animal assay system which provides a model system for testing for an agent that reduces or inhibits an OB-BPL associated pathology

(a) administering the agent to a transgenic non-human animal according to claim 25; and

(b) determining whether said agent reduces or inhibits an OB-BPL associated pathology in the transgenic non-human animal relative to a transgenic non-human animal of step (a) which has not been administered the agent.

Kindly add new claims 27-40 as follows.

27. A regulatory sequence of an isolated nucleic acid molecule of claim 1 fused to a nucleic acid which encodes a heterologous protein.

28. A vector comprising a nucleic acid molecule of claim 1.

29. A host cell comprising a nucleic acid molecule of claim 1.

30. A method for preparing a protein comprising:

(a) transferring a vector of claim 29 into a host cell;

(b) selecting transformed host cells from untransformed host cells;

(c) culturing a selected transformed host cell under conditions which allow expression of the protein; and

(d) isolating the protein.

31. A method of diagnosing and monitoring conditions mediated by a OB-BPL protein by determining the presence of a nucleic acid molecule of claim 1 or a polypeptide selected from the group consisting of a polypeptide comprising an amino acid sequence of SEQ. ID. NO. 2 or 3 and a polypeptide having at least 65% amino acid sequence identity to an amino acid sequence of SEQ. ID. NO. 2 or 3.

32. A method for treating a condition mediated by an OB-BPL protein comprising administering an effective amount of an antibody having specificity against a protein epitope of claim 8.

33. A method for treating a condition mediated by an OB-BPL protein comprising administering an effective amount of a substance or compound identified by the method of claim 17.

34. A method for treating a condition mediated by an OB-BPL protein comprising administering an effective amount of a substance or compound identified by the method of claim 18.

35. A method according to claim 33, wherein the condition is a disorder of the hematopoietic system.

36. A method according to claim 34, wherein the condition is a disorder of the hematopoietic system.

37. A composition comprising a nucleic acid molecule of claim 1 and a pharmaceutically acceptable carrier, excipient or diluent.

38. A composition comprising a protein of claim 8 and a pharmaceutically acceptable carrier, excipient or diluent.

39. A composition comprising a substance or compound identified by the method of claim 17 and a pharmaceutically acceptable carrier, excipient or diluent.

40. A composition comprising a substance or compound identified by the method of claim 18 and a pharmaceutically acceptable carrier, excipient or diluent.

REMARKS

Upon entry of this second preliminary amendment, claims 1-4, 8, 11-14, 16-20, and 25-40 are in this application. New claims 27-40 are supported throughout the specification and by original claims 5-7, 9-10, 15, and 21-24, respectively, and have eliminated multiple dependencies. No new matter is added by this preliminary amendment.

The attached Abstract of the Disclosure is supported throughout the specification.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached Appendix A is captioned "**Version With Markings to Show Changes Made**".

Attached hereto is a clean copy of all of the pending claims. The attached Appendix B is captioned "**Clean Copy of Pending Claims Without Markings**".

Applicants respectfully request consideration of the pending claims.

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to our Deposit Account No. 08-3040.

Respectfully submitted,
HOWSON AND HOWSON
Attorneys for the Applicants

By ______________________

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Telefacsimile: (215) 540-5818

ABSTRACT OF THE DISCLOSURE

The invention relates to nucleic acid molecules, proteins encoded by such nucleic acid molecules; and use of the proteins and nucleic acid molecules.

Appendix A

Version with Markings to Show Changes Made

<u>In the specification:</u>

Page 1, line 2, before "Field of the Invention", insert the following new paragraph:

-- Cross-Reference to Related Applications

This is a 371 of PCT/CA00/00259, filed March 9, 2000, which claims the benefit of the priorities of U. S. Patent Application No. 60/144,919, filed July 21, 1999, now abandoned, U. S. Patent Application No. 60/127,386, filed April 1, 1999, now abandoned, and U. S. Patent Application No. 60/124,260, filed March 11, 1999, now abandoned. --

<u>In the claims:</u>

Claim 2 has been amended as follows:

2. (Amended) ~~An~~ <u>The</u> isolated nucleic acid molecule <u>according to claim 1</u> which comprises:

(i) a nucleic acid sequence encoding a polypeptide having substantial sequence identify with the amino acid sequence shown in Table 5 or SEQ ID NO: 2 or SEQ ID NO: 3;

(ii) nucleic acid sequences complementary to (i);

(iii) a degenerate form of a nucleic acid sequence of (i);

(iv) a nucleic acid sequence comprising at least 18 nucleotides and capable of hybridizing to a nucleic acid sequence in (i), (ii), or (iii);

(v) a nucleic acid sequence encoding a truncation, an analog, an allelic or species variation of a polypeptide comprising the amino acid sequence shown in Table 5 or SEQ ID NO: 2, or SEQ ID NO: 3; or

(vi) a fragment, or allelic or species variation of (i), (ii) or (iii).

Claim 3 has been amended as follows:

3. (Amended) ~~An~~ The isolated nucleic acid molecule according to claim 1 which comprises:

(a) a nucleic acid sequence having substantial sequence identity or sequence similarity with a nucleic acid sequence of SEQ ID NO: 1;

(b) nucleic acid sequences complementary to (i), preferably complementary to the full nucleic acid sequence of SEQ ID NO: 1;

(c) nucleic acid sequences differing from any of the nucleic acid sequences of (i) or (ii) in codon sequences due to the degeneracy of the genetic code; or

(d) a fragment, or allelic or species variation of (i), (ii) or (iii).

Claim 8 has been amended as follows:

8. (Amended) An isolated ~~OB-BPL~~ protein selected from the group consisting of a protein comprising an amino acid sequence of SEQ. ID. NO. 2 or 3 and a protein having at least 65% amino acid sequence identity to an amino acid sequence of SEQ. ID. NO. 2 or 3.

Claim 11 has been amended as follows:

11. (Amended) A protein prepared in accordance with the method of claim ~~11~~ 30.

Claim 12 has been amended as follows:

12. (Amended) An antibody having specificity against an epitope of a polypeptide ~~as claimed in claim 9~~ of claim 8.

Claim 13 has been amended as follows:

13. (Amended) An antibody ~~as claimed in~~ according to claim ~~13~~ 12 labeled with a detectable substance and used to detect the polypeptide in biological samples, tissues, and cells.

Claim 14 has been amended as follows:

14. (Amended) A probe comprising a sequence encoding a protein ~~as claimed in~~ according to claim ~~9~~ 8, or a part thereof.

Claim 16 has been amended as follows:

16. (Amended) A method ~~as claimed in~~ according to claim ~~16~~ 31 wherein the condition is cancer or a disorder of the hematopoietic system.

Claim 17 has been amended as follows:

17. (Amended) A method for identifying a substance which associates with a protein ~~as claimed in~~ according to claim ~~9~~ 8 comprising (a) reacting the protein with at least one substance which potentially can associate with the protein, under conditions which permit the association between the substance and protein, and (b) removing or detecting protein associated with the substance, wherein detection of associated protein and substance indicates the substance associates with the protein.

Claim 18 has been amended as follows:

18. (Amended) A method for evaluating a compound for its ability to modulate the biological activity of a protein ~~as claimed in~~ according to claim ~~9~~ 8 comprising providing a known concentration of the protein with a substance which associates with the protein and a test compound under conditions which permit the formation of complexes between the substance and protein, and removing and/or detecting complexes.

Claim 20 has been amended as follows:

20. (Amended) A method ~~as claimed in~~ according to claim ~~20~~ 19 wherein nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

Claim 25 has been amended as follows:

25. (Amended) A transgenic non-human mammal which does not express an OB-BPL protein ~~as claimed in~~ according to claim ~~9~~ 8 resulting in an OB-BPL associated pathology.

Claim 26 has been amended as follows:

26. (Amended) A transgenic animal assay system which provides a model system for testing for an agent that reduces or inhibits an OB-BPL associated pathology

(a) administering the agent to a transgenic non-human animal ~~as claimed in~~ according to claim ~~26~~ 25; and

(b) determining whether said agent reduces or inhibits an OB-BPL associated pathology in the transgenic non-human animal relative to a transgenic non-human animal of step (a) which has not been administered the agent.

Appendix B

Clean Copy of Pending Claims Without Markings

1. An isolated OB-BPL nucleic acid molecule of at least 30 nucleotides which hybridizes to SEQ ID NO: 1, or the complement of SEQ ID NO: 1, under stringent hybridization conditions.

2. The isolated nucleic acid molecule according to claim 1 which comprises:

(i) a nucleic acid sequence encoding a polypeptide having substantial sequence identify with the amino acid sequence shown in Table 5 or SEQ ID NO: 2 or SEQ ID NO: 3;

(ii) nucleic acid sequences complementary to (i);

(iii) a degenerate form of a nucleic acid sequence of (i);

(iv) a nucleic acid sequence comprising at least 18 nucleotides and capable of hybridizing to a nucleic acid sequence in (i), (ii), or (iii);

(v) a nucleic acid sequence encoding a truncation, an analog, an allelic or species variation of a polypeptide comprising the amino acid sequence shown in Table 5 or SEQ ID NO: 2, or SEQ ID NO: 3; or

(vi) a fragment, or allelic or species variation of (i), (ii) or (iii).

3. The isolated nucleic acid molecule according to claim 1 which comprises:

(a) a nucleic acid sequence having substantial sequence identity or sequence similarity with a nucleic acid sequence of SEQ ID NO: 1;

(b) nucleic acid sequences complementary to (i), preferably complementary to the full nucleic acid sequence of SEQ ID NO: 1;

(c) nucleic acid sequences differing from any of the nucleic acid sequences of (i) or (ii) in codon sequences due to the degeneracy of the genetic code; or

(d) a fragment, or allelic or species variation of (i), (ii) or (iii).

4. An isolated nucleic acid molecule which encodes a protein which binds an antibody of a OB-BPL polypeptide.

8. An isolated protein selected from the group consisting of a protein comprising an amino acid sequence of SEQ. ID. NO. 2 or 3 and a protein having at least 65% amino acid sequence identity to an amino acid sequence of SEQ. ID. NO. 2 or 3.

11. A protein prepared in accordance with the method of claim 30.

12. An antibody having specificity against an epitope of a polypeptide of claim 8.

13. An antibody according to claim 12 labeled with a detectable substance and used to detect the polypeptide in biological samples, tissues, and cells.

14. A probe comprising a sequence encoding a protein according to claim 8, or a part thereof.

16. A method according to claim 31 wherein the condition is cancer or a disorder of the hematopoietic system.

17. A method for identifying a substance which associates with a protein according to claim 8 comprising (a) reacting the protein with at least one substance which potentially can associate with the protein, under conditions which permit the association between the substance and protein, and (b) removing or detecting protein associated with the substance, wherein detection of associated protein and substance indicates the substance associates with the protein.

18. A method for evaluating a compound for its ability to modulate the biological activity of a protein according to claim 8 comprising providing a known concentration of the protein with a substance which associates with the protein and a test compound under conditions which permit the formation of complexes between the substance and protein, and removing and/or detecting complexes.

19. A method for detecting a nucleic acid molecule encoding a protein comprising an amino acid sequence of SEQ ID NO: 2 or 3 in a biological sample comprising the steps of:

(a) hybridizing a nucleic acid molecule of claim 2 to nucleic acids of the biological sample, thereby forming a hybridization complex; and

(b) detecting the hybridization complex wherein the presence of the hybridization complex correlates with the presence of a nucleic acid molecule encoding the protein in the biological sample.

20. A method according to claim 19 wherein nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

25. A transgenic non-human mammal which does not express an OB-BPL protein according to claim 8 resulting in an OB-BPL associated pathology.

26. A transgenic animal assay system which provides a model system for testing for an agent that reduces or inhibits an OB-BPL associated pathology

(a) administering the agent to a transgenic non-human animal according to claim 25; and

(b) determining whether said agent reduces or inhibits an OB-BPL associated pathology in the transgenic non-human animal relative to a transgenic non-human animal of step (a) which has not been administered the agent.

27. A regulatory sequence of an isolated nucleic acid molecule of claim 1 fused to a nucleic acid which encodes a heterologous protein.

28. A vector comprising a nucleic acid molecule of claim 1.

29. A host cell comprising a nucleic acid molecule of claim 1.

30. A method for preparing a protein comprising:

(a) transferring a vector of claim 29 into a host cell;

(b) selecting transformed host cells from untransformed host cells;

(c) culturing a selected transformed host cell under conditions which allow expression of the protein; and

(d) isolating the protein.

31. A method of diagnosing and monitoring conditions mediated by a OB-BPL protein by determining the presence of a nucleic acid molecule of claim 1 or a polypeptide selected from the group consisting of a polypeptide comprising an amino acid sequence of SEQ. ID. NO. 2 or 3 and a polypeptide having at least 65% amino acid sequence identity to an amino acid sequence of SEQ. ID. NO. 2 or 3.

32. A method for treating a condition mediated by an OB-BPL protein comprising administering an effective amount of an antibody having specificity against a protein epitope of claim 8.

33. A method for treating a condition mediated by an OB-BPL protein comprising administering an effective amount of a substance or compound identified by the method of claim 17.

34. A method for treating a condition mediated by an OB-BPL protein comprising administering an effective amount of a substance or compound identified by the method of claim 18.

35. A method according to claim 33, wherein the condition is a disorder of the hematopoietic system.

36. A method according to claim 34, wherein the condition is a disorder of the hematopoietic system.

37. A composition comprising a nucleic acid molecule of claim 1 and a pharmaceutically acceptable carrier, excipient or diluent.

38. A composition comprising a protein of claim 8 and a pharmaceutically acceptable carrier, excipient or diluent.

39. A composition comprising a substance or compound identified by the method of claim 17 and a pharmaceutically acceptable carrier, excipient or diluent.

40. A composition comprising a substance or compound identified by the method of claim 18 and a pharmaceutically acceptable carrier, excipient or diluent.

MTS4USA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	) Group Art Unit:
	)
George Foussias et al	) Examiner:
	)
Appln. No.	)
	)
Filed: Herewith	)
	)
For: SIALIC ACID-BINDING IG-LIKE LECTIN (SIGLEC) GENE; OB-BINDING PROTEIN LIKE (OB-BPL)	) September 10, 2001))

Assistant Commissioner for Patents
Box PCT
Washington, DC 20231

PRELIMINARY AMENDMENT A

Sir:

Please amend the above-identified patent application as follows.

In the Claims

Cancel claims 5-7, 9-10, 15, and 21-24 without prejudice.

Express Mail No. ET033648209US

REMARKS

After entry of this preliminary amendment, the pending claims are claims 1-4, 8, 11-14, 16-20, and 25-26. Claims 5-7, 9-10, 15, and 21-24 are canceled. No new matter is introduced by this preliminary amendment.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version With Markings to Show Changes Made**".

Applicants respectfully request that this preliminary amendment be entered prior to calculating the filing fees.

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to Deposit Account No. 08-3040.

Respectfully submitted,

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Appendix A

Version with Markings to Show Changes Made

In the claims:

Claims 5-7, 9-10, 15, and 21-24 have been cancelled.

SIALIC ACID–BINDING IG–LIKE LECTIN (SIGLEC) GENE; OB–BINDING PROTEIN LIKE (OB–BPL)

FIELD OF THE INVENTION

The invention relates to nucleic acid molecules, proteins encoded by such nucleic acid molecules; and use of the proteins and nucleic acid molecules

BACKGROUND OF THE INVENTION

The immunoglobulin superfamily (IgSF) encompasses a large number of cell surface molecules which play a vital role not only in immunity, but also in controlling the behaviour of cells in various tissues, through their ability to mediate cell surface recognition events. These molecules are characterized by the presence of at least one immunoglobulin (Ig) domain, a sandwich of two β-sheets stabilized by a conserved disulfide bond. The core of this domain is composed of β-strands A,B,E in one sheet and G,F,C in the other, and arise from the ends of the domain sequence (Williams and Barclay 1988). In between, however, there is a great deal of sequence length variation. Such Ig domains occur in two types, the V-set and the C-set, and can be distinguished based on patterns of conserved amino acid residues responsible for forming the characteristic β-sheet sandwich. V-set domains consist of about 65-75 amino acid residues between conserved cysteines, whereas C-set domains have about 55-60 residues (reviewed in (Williams and Barclay 1988)). The C-set domains can be further divided into C1- and C2-sets, and are distinguished by the fact that, although showing signs of a C-set domain, the latter half of C2-set domains exhibit sequence patterns more homologous to V-set rather than C1-set domains (Williams *et al.*, 1989).

Recently, a novel family of structurally related IgSF molecules have been identified, which mediate protein-carbohydrate interactions through specific interactions with sialic acid-containing glycoproteins and glycolipids (Crocker *et al.*, 1996). This family was originally referred to as the sialoadhesins, but has recently been designated the sialic acid-binding Ig-like lectin (Siglec) family (Crocker *et al.*, 1998). These molecules are characterized by the presence of one N-terminal V-set domain, and a variable number of downstream C2-set domains, ranging from 16 in sialoadhesin to 1 in CD33 (Crocker *et al.*, 1996). Furthermore, these Ig-like domains possess some unique features. In the V-set domain, the conserved cysteine in β-strand F of classic V-set domains is absent, while a highly conserved cysteine is present in β-strand E in all siglecs identified so far. This results in the cysteines in β-strands B and E being next to each other in one β-sheet, which likely results in an intrasheet disulfide bond (Crocker *et al.*, 1996; Williams *et al.*, 1989). There is also an additional highly conserved cysteine residue in both the V-set and first C2-set domains of all siglecs. In the V-set domain it is located at the beginning of β-strand B, while in the C2-set domain it is found between β-strands B and C. These two additional cysteines have been found to form an interdomain disulfide bond, a feature unique to siglecs (Crocker *et al.*, 1996; Pedraza *et al.*, 1990).

Currently, the siglec family consists of sialoadhesin (Siglec-1), CD22 (Siglec-2), CD33 (Siglec-3), myelin-associated glycoprotein (MAG) (Siglec-4a), Schwann cell myelin protein (SMP) (Siglec-4b), OB-binding protein 2 (Siglec-5), OB-binding protein 1 (Siglec-6), and p75/AIRM1 (Siglec-7) (Cornish *et al.*, 1998; Crocker *et al.*, 1998; Falco *et al.*, 1999; Nicoll *et al.*, 1999; Patel *et al.*, 1999). Each member of the

Siglec family is expressed by specific cell types and exhibits a distinct function. Sialoadhesin is a macrophage-restricted adhesion molecule (Crocker *et al.*, 1994), CD22 is B lymphocyte-specific and regulates its activation (Stamenkovic and Seed 1990), CD33 is a myeloid-specific inhibitory receptor (Ulyanova *et al.*, 1999), and MAG functions in the formation and maintenance of axonal myelin structure (Li *et al.*, 1998). Siglec-5 and –6 (OB-BP2 and -BP1, respectively) are expressed in several tissues including placenta and peripheral blood leukocytes, and have shown an *in vitro* ability to bind leptin (Cornish *et al.*, 1998; Patel *et al.*, 1999), while OB-BPL (p75/AIRM1) is an inhibitory receptor expressed predominantly on human natural killer cells (Falco *et al.*, 1999; Nicoll *et al.*, 1999).

SUMMARY OF THE INVENTION

The present inventors have identified and characterized a gene encoding a novel member of the siglec family (OB-binding protein like or OB-BPL). The putative protein product displays a high degree of homology with siglec-7, as well as with siglec-5 and siglec-6. Further, it possesses all the structural features found in other siglecs. The gene was localized to 19q13.4, 43.19 Kb more telomeric than KLK-L6 (a member of the kallikrein gene family) through genomic sequencing data and restriction mapping with EcoRI. The novel siglec is encoded by 7 exons, with six intervening introns. In addition, it is highly expressed in bone marrow, placenta, spleen, and fetal liver, as well as other tissues at lower levels.

The OB-BPL protein described herein is referred to as "OB-BPL Protein". The gene encoding the protein is referred to as "*ob-bpl*".

Broadly stated the present invention relates to an isolated nucleic acid molecule which comprises:

(i) a nucleic acid sequence encoding a protein having substantial sequence identity with an amino acid sequence of OB-BPL as shown in Table 5 or SEQ.ID.NO. 2 or 3;

(ii) a nucleic acid sequence encoding a protein comprising an amino acid sequence of OB-BPL as shown in Table 5 or SEQ.ID.NO. 2 or 3;

(iii) nucleic acid sequences complementary to (i);

(iv) a degenerate form of a nucleic acid sequence of (i);

(v) a nucleic acid sequence capable of hybridizing under stringent conditions to a nucleic acid sequence in (i), (ii) or (iii);

(vi) a nucleic acid sequence encoding a truncation, an analog, an allelic or species variation of a protein comprising an amino acid sequence of OB-BPL as shown in Table 5 or SEQ.ID.NO. 2 or 3; or

(vii) a fragment, or allelic or species variation of (i), (ii) or (iii).

Preferably, a purified and isolated nucleic acid molecule of the invention comprises:

(i) a nucleic acid sequence comprising the sequence of SEQ.ID.NO. 1 wherein T can also be U;

(ii) nucleic acid sequences complementary to (i), preferably complementary to the full nucleic acid sequence of SEQ.ID.NO. 1;

(iii) a nucleic acid capable of hybridizing under stringent conditions to a nucleic acid of (i) or (ii) and preferably having at least 18 nucleotides; or

(iv) a nucleic acid molecule differing from any of the nucleic acids of (i) to (iii) in codon sequences due to the degeneracy of the genetic code.

The invention also contemplates a nucleic acid molecule comprising a sequence encoding a truncation of an OB-BPL Protein, an analog, or a homolog of an OB-BPL Protein or a truncation thereof. (OB-BPL Protein and truncations, analogs and homologs of OB-BPL Protein are also collectively referred to herein as " OB-BPL Related Proteins").

The nucleic acid molecules of the invention may be inserted into an appropriate expression vector, i.e. a vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Accordingly, recombinant expression vectors adapted for transformation of a host cell may be constructed which comprise a nucleic acid molecule of the invention and one or more transcription and translation elements linked to the nucleic acid molecule.

The recombinant expression vector can be used to prepare transformed host cells expressing OB-BPL Related Proteins. Therefore, the invention further provides host cells containing a recombinant molecule of the invention. The invention also contemplates transgenic non-human mammals whose germ cells and somatic cells contain a recombinant molecule comprising a nucleic acid molecule of the invention, in particular one which encodes an analog of the OB-BPL Protein, or a truncation of the OB-BPL Protein.

The invention further provides a method for preparing OB-BPL Related Proteins utilizing the purified and isolated nucleic acid molecules of the invention. In an embodiment a method for preparing an OB-BPL Related Protein is provided comprising (a) transferring a recombinant expression vector of the invention into a host cell; (b) selecting transformed host cells from untransformed host cells; (c) culturing a selected transformed host cell under conditions which allow expression of the OB-BPL Related Protein; and (d) isolating the OB-BPL Related Protein.

The invention further broadly contemplates an isolated OB-BPL Protein comprising an amino acid sequence as shown in SEQ.ID.NO. 2 or 3.

The OB-BPL Related Proteins of the invention may be conjugated with other molecules, such as proteins, to prepare fusion proteins. This may be accomplished, for example, by the synthesis of N-terminal or C-terminal fusion proteins.

The invention further contemplates antibodies having specificity against an epitope of an OB-BPL Related Protein of the invention. Antibodies may be labeled with a detectable substance and used to detect proteins of the invention in tissues and cells.

The invention also permits the construction of nucleotide probes which are unique to the nucleic acid molecules of the invention and/or to proteins of the invention. Therefore, the invention also relates to a probe comprising a nucleic acid sequence of the invention, or a nucleic acid sequence encoding a protein of the invention, or a part thereof. The probe may be labeled, for example, with a detectable substance and it may be used to select from a mixture of nucleotide sequences a nucleic acid molecule of the invention including nucleic acid molecules coding for a protein which displays one or more of the properties of a protein of the invention.

The invention still further provides a method for identifying a substance which binds to a protein

of the invention comprising reacting the protein with at least one substance which potentially can bind with the protein, under conditions which permit the formation of complexes between the substance and protein and detecting binding. Binding may be detected by assaying for complexes, for free substance, or for non-complexed protein. The invention also contemplates methods for identifying substances that bind to other intracellular proteins that interact with an OB-BPL Related Protein. Methods can also be utilized which identify compounds which bind to OB-BPL gene regulatory sequences (e.g. promoter sequences).

Still further the invention provides a method for evaluating a compound for its ability to modulate the biological activity of an OB-BPL Related Protein of the invention. For example a substance which inhibits or enhances the interaction of the protein and a substance which binds to the protein may be evaluated. In an embodiment, the method comprises providing a known concentration of an OB-BPL Related Protein, with a substance which binds to the protein and a test compound under conditions which permit the formation of complexes between the substance and protein, and removing and/or detecting complexes.

Compounds which modulate the biological activity of a protein of the invention may also be identified using the methods of the invention by comparing the pattern and level of expression of the protein of the invention in tissues and cells, in the presence, and in the absence of the compounds.

The proteins of the invention and substances and compounds identified using the methods of the invention, and peptides of the invention may be used to modulate the biological activity of an OB-BPL Related Protein of the invention, and they may be used in the treatment of conditions such a disorders of the hematopoietic system and in particular leukemias. Accordingly, the substances and compounds may be formulated into compositions for administration to individuals suffering from a disorders of the hematopoietic system.

Therefore, the present invention also relates to a composition comprising one or more of a protein of the invention, a peptide of the invention, or a substance or compound identified using the methods of the invention, and a pharmaceutically acceptable carrier, excipient or diluent. A method for treating or preventing cancer or a disorder of the hematopoietic system is also provided comprising administering to a patient in need thereof, an OB-BPL Related Protein of the invention, or a composition of the invention.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1: Genomic Structure of a Novel Siglec. Shown are the exon/intron boundaries, as well as the predicted protein sequence. The single underlined region is the 5' untranslated region, and the double underlined region is the 3' untranslated region. In the shaded box is the putative polyadenylation signal.

Figure 2: Hydophobicity Plot of the Novel Siglec. This shows the regions of the putative novel siglec protein which contain stretches of hydrophobic amino acid residues. As is evident, there are two such regions, the first corresponding to the signal peptide, and the second, at around residues 350-370, the putative transmembrane region.

Figure 3: Localization of the Novel Siglec Gene. The physical map of the genomic area around chromosome 19q13.3-q13.4 where the kallikrein gene family resides. Seven additional kallikreins map in the 132.1 Kb region (data not shown; see (Diamandis *et al.*, 1999)). Gene lengths are presented above each arrow, and distances between genes are shown below. Arrows denote the direction of transcription. The novel siglec gene resides 43.2 Kb telomeric to the KLK-L6 gene. KLK, kallikrein; PSA, prostate specific antigen; KLK-L, kallikrein-like; NES1, normal epithelial cell-specific 1 gene; TLSP, trypsin-like serine protease.

Figure 4: Siglec Family Multiple Alignment. The novel siglec was aligned with siglec-5 to –7 and CD33, using ClustalX (Jeanmougin *et al.*, 1998) (SEQ. ID. NOs. 10-13). The signal peptide was determined through computer prediction, and the Ig domain boundaries were assigned based on exon boundaries. The transmembrane domain was also predicted, while taking into consideration exon boundaries as well. The ITIM-like and SLAM-like motifs are indicated, as are the conserved cysteines (*) which form the disulfide bonds of the Ig-like domains in siglecs, and the conserved arginine and aromatic residues (¤) which are responsible for sialic acid binding and specificity.

Figure 5: Phylogenetic Analysis of the Siglec Family. The phylogenetic tree was created using ClustalX (Jeanmougin *et al.*, 1998) and TreeView (Page 1996). As is evident, siglec-7 and the novel siglec are very closely related, and they are both related to CD33, in addition to a more distant relation to the other siglecs.

Figure 6: Tissue Expression Profile of the Novel Siglec. RT-PCR was performed on 28 tissue total RNAs, for this novel siglec and actin (control gene). The novel siglec is highly expressed in bone marrow, placenta, spleen, and fetal liver. There is also a lower degree of expression in many of the other tissues, while it is absent in ovary, pancreas, skeletal muscle, and heart.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See for example, Sambrook, Fritsch, & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y); DNA Cloning: A Practical Approach, Volumes I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M..J. Gait ed. 1984); Nucleic Acid Hybridization B.D. Hames & S.J. Higgins eds. (1985); Transcription and Translation B.D. Hames & S.J. Higgins eds (1984); Animal Cell Culture R.I. Freshney, ed. (1986); Immobilized Cells and enzymes IRL Press, (1986); and B. Perbal, A Practical Guide to Molecular Cloning (1984).

1. Nucleic Acid Molecules of the Invention

As hereinbefore mentioned, the invention provides an isolated nucleic acid molecule having a

sequence encoding an OB-BPL Protein. The term "isolated" refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical reactants, or other chemicals when chemically synthesized. An "isolated" nucleic acid may also be free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid molecule) from which the nucleic acid is derived. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded. In an embodiment, a nucleic acid molecule encodes an OB-BPL Protein comprising an amino acid sequence as shown in SEQ.ID.NO. 2 or 3, preferably a nucleic acid molecule comprising a nucleic acid sequence as shown in SEQ.ID.NO. 1.

The invention includes nucleic acid sequences complementary to a nucleic acid encoding an OB-BPL Protein comprising an amino acid sequence as shown in SEQ.ID.NO. 2 or 3, preferably the nucleic acid sequences complementary to a full nucleic acid sequence shown in SEQ.ID.NO. 1.

The invention includes nucleic acid molecules having substantial sequence identity or homology to nucleic acid sequences of the invention or encoding proteins having substantial identity or similarity to the amino acid sequence shown in in SEQ.ID.NO. 2 or 3. Preferably, the nucleic acids have substantial sequence identity for example at least 65%, 70%, 75%, 80%, or 85% nucleic acid identity; more preferably 90% nucleic acid identity; and most preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity. "Identity" as known in the art and used herein, is a relationship between two or more amino acid sequences or two or more nucleic acid sequences, as determined by comparing the sequences. It also refers to the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. Identity and similarity are well known terms to skilled artisans and they can be calculated by conventional methods (for example see Computational Molecular Biology, Lesk, A.M. ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W. ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M. and Griffin, H.G. eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G. Acadmeic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J. eds. M. Stockton Press, New York, 1991, Carillo, H. and Lipman, D., SIAM J. Applied Math. 48:1073, 1988). Methods which are designed to give the largest match between the sequences are generally preferred. Methods to determine identity and similarity are codified in publicly available computer programs including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990).

Isolated nucleic acid molecules encoding an OB-BPL Protein, and having a sequence which differs from a nucleic acid sequence of the invention due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent proteins (e.g., an OB-BPL Protein) but differ in sequence from the sequence of an OB-BPL Protein due to degeneracy in the genetic code. As one example, DNA sequence polymorphisms within the nucleotide sequence of an OB-BPL Protein may result in silent mutations which do not affect the amino acid sequence. Variations in one or

more nucleotides may exist among individuals within a population due to natural allelic variation. Any and all such nucleic acid variations are within the scope of the invention. DNA sequence polymorphisms may also occur which lead to changes in the amino acid sequence of an OB-BPL Protein. These amino acid polymorphisms are also within the scope of the present invention.

Another aspect of the invention provides a nucleic acid molecule which hybridizes under stringent conditions, preferably high stringency conditions to a nucleic acid molecule which comprises a sequence which encodes an OB-BPL Protein having an amino acid sequence shown in SEQ.ID.NO. 2 or 3. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

It will be appreciated that the invention includes nucleic acid molecules encoding an OB-BPL Related Protein including truncations of an OB-BPL Protein, and analogs of an OB-BPL Protein as described herein. The truncated nucleic acids or nucleic acid fragments may correspond to the transmembrane domain, cytoplasmic domain, IG domains,or ITIM-like or SLAM-like motifs as described in Table 4 and in Figure 4. It will further be appreciated that variant forms of the nucleic acid molecules of the invention which arise by alternative splicing of an mRNA corresponding to a cDNA of the invention are encompassed by the invention.

An isolated nucleic acid molecule of the invention which comprises DNA can be isolated by preparing a labelled nucleic acid probe based on all or part of a nucleic acid sequence of the invention. The labeled nucleic acid probe is used to screen an appropriate DNA library (e.g. a cDNA or genomic DNA library). For example, a cDNA library can be used to isolate a cDNA encoding an OB-BPL Related Protein by screening the library with the labeled probe using standard techniques. Alternatively, a genomic DNA library can be similarly screened to isolate a genomic clone encompassing a gene encoding an OB-BPL Related Protein. Nucleic acids isolated by screening of a cDNA or genomic DNA library can be sequenced by standard techniques.

An isolated nucleic acid molecule of the invention which is DNA can also be isolated by selectively amplifying a nucleic acid encoding an OB-BPL Related Protein using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the nucleotide sequence of the invention for use in PCR. A nucleic acid can be amplified from cDNA or genomic DNA using these oligonucleotide primers and standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., Biochemistry, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example,

Deletions may consist of the removal of one or more amino acids, or discrete portions from an OB-BPL Protein sequence. The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably 20 to 40 amino acids.

The proteins of the invention include proteins with sequence identity or similarity to an OB-BPL Protein and/or truncations thereof as described herein. Such OB-BPL Proteins include proteins whose amino acid sequences are comprised of the amino acid sequences of OB-BPL Protein regions from other species that hybridize under selected hybridization conditions (see discussion of stringent hybridization conditions herein) with a probe used to obtain an OB-BPL Protein. These proteins will generally have the same regions which are characteristic of an OB-BPL Protein. Preferably a protein will have substantial sequence identity for example, about 65%, 70%, 75%, 80%, or 85% identity, preferably 90% identity, more preferably at least 95%, 96%, 97%, 98%, or 99% identity, and most preferably 98% identity with an amino acid sequence shown in in SEQ.ID.NO. 2 or 3. A percent amino acid sequence homology, similarity or identity is calculated as the percentage of aligned amino acids that match the reference sequence using known methods as described herein.

The invention also contemplates isoforms of the proteins of the invention. An isoform contains the same number and kinds of amino acids as a protein of the invention, but the isoform has a different molecular structure. Isoforms contemplated by the present invention preferably have the same properties as a protein of the invention as described herein.

The present invention also includes OB-BPL Related Proteins conjugated with a selected protein, or a marker protein (see below) to produce fusion proteins. Additionally, immunogenic portions of an OB-BPL Protein and an OB-BPL Protein Related Protein are within the scope of the invention.

AN OB-BPL Related Protein of the invention may be prepared using recombinant DNA methods. Accordingly, the nucleic acid molecules of the present invention having a sequence which encodes an OB-BPL Related Protein of the invention may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence. Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes [For example, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990)]. Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. The necessary regulatory sequences may be supplied by the native OB-BPL Protein and/or its flanking regions.

The invention further provides a recombinant expression vector comprising a DNA nucleic acid molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is linked to a regulatory sequence in a manner which allows for expression, by transcription of

the DNA molecule, of an RNA molecule which is antisense to the nucleic acid sequence of a protein of the invention or a fragment thereof. Regulatory sequences linked to the antisense nucleic acid can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance a viral promoter and/or enhancer, or regulatory sequences can be chosen which direct tissue or cell type specific expression of antisense RNA.

The recombinant expression vectors of the invention may also contain a marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β-galactosidase, chloramphenicol acetyltransferase, firefly luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. The markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

The recombinant expression vectors may be introduced into host cells to produce a transformant host cell. "Transformant host cells" include host cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" encompass the introduction of a nucleic acid (e.g. a vector) into a cell by one of many standard techniques. Prokaryotic cells can be transformed with a nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. A nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells, or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991).

A host cell may also be chosen which modulates the expression of an inserted nucleic acid sequence, or modifies (e.g. glycosylation or phosphorylation) and processes (e.g. cleaves) the protein in a desired fashion. Host systems or cell lines may be selected which have specific and characteristic mechanisms for post-translational processing and modification of proteins. For example, eukaryotic host

cells including CHO, VERO, BHK, HeLA, COS, MDCK, 293, 3T3, and WI38 may be used. For long-term high-yield stable expression of the protein, cell lines and host systems which stably express the gene product may be engineered.

Host cells and in particular cell lines produced using the methods described herein may be particularly useful in screening and evaluating compounds that modulate the activity of an OB-BPL Related Protein.

The proteins of the invention may also be expressed in non-human transgenic animals including but not limited to mice, rats, rabbits, guinea pigs, micro-pigs, goats, sheep, pigs, non-human primates (e.g. baboons, monkeys, and chimpanzees) [see Hammer et al. (Nature 315:680-683, 1985), Palmiter et al. (Science 222:809-814, 1983), Brinster et al. (Proc Natl. Acad. Sci USA 82:44384442, 1985), Palmiter and Brinster (Cell. 41:343-345, 1985) and U.S. Patent No. 4,736,866)]. Procedures known in the art may be used to introduce a nucleic acid molecule of the invention encoding an OB-BPL Related Protein into animals to produce the founder lines of transgenic animals. Such procedures include pronuclear microinjection, retrovirus mediated gene transfer into germ lines, gene targeting in embryonic stem cells, electroporation of embryos, and sperm-mediated gene transfer.

The present invention contemplates a transgenic animal that carries the *OB-BPL* gene in all their cells, and animals which carry the transgene in some but not all their cells. The transgene may be integrated as a single transgene or in concatamers. The transgene may be selectively introduced into and activated in specific cell types (See for example, Lasko et al, 1992 Proc. Natl. Acad. Sci. USA 89: 6236). The transgene may be integrated into the chromosomal site of the endogenous gene by gene targeting. The transgene may be selectively introduced into a particular cell type inactivating the endogenous gene in that cell type (See Gu et al Science 265: 103-106).

The expression of a recombinant OB-BPL Related Protein in a transgenic animal may be assayed using standard techniques. Initial screening may be conducted by Southern Blot analysis, or PCR methods to analyze whether the transgene has been integrated. The level of mRNA expression in the tissues of transgenic animals may also be assessed using techniques including Northern blot analysis of tissue samples, *in situ* hybridization, and RT-PCR. Tissue may also be evaluated immunocytochemically using antibodies against OB-BPL Protein.

Proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart).

N-terminal or C-terminal fusion proteins comprising an OB-BPL Related Protein of the invention conjugated with other molecules, such as proteins, may be prepared by fusing, through recombinant techniques, the N-terminal or C-terminal of an OB-BPL Related Protein, and the sequence of a selected protein or marker protein with a desired biological function. The resultant fusion proteins contain OB-BPL Protein fused to the selected protein or marker protein as described herein. Examples of proteins which may be used to prepare fusion proteins include immunoglobulins, glutathione-S-transferase (GST),

hemagglutinin (HA), and truncated myc.

3. **Antibodies**

OB-BPL Related Proteins of the invention can be used to prepare antibodies specific for the proteins. Antibodies can be prepared which bind a distinct epitope in an unconserved region of the protein. An unconserved region of the protein is one that does not have substantial sequence homology to other proteins. A region from a conserved region such as a well-characterized domain can also be used to prepare an antibody to a conserved region of an OB-BPL Related Protein. Antibodies having specificity for an OB-BPL Related Protein may also be raised from fusion proteins created by expressing fusion proteins in bacteria as described herein.

The invention can employ intact monoclonal or polyclonal antibodies, and immunologically active fragments (e.g. a Fab, $(Fab)_2$ fragment, or Fab expression library fragments and epitope-binding fragments thereof), an antibody heavy chain, and antibody light chain, a genetically engineered single chain Fv molecule (Ladner et al, U.S. Pat. No. 4,946,778), or a chimeric antibody, for example, an antibody which contains the binding specificity of a murine antibody, but in which the remaining portions are of human origin. Antibodies including monoclonal and polyclonal antibodies, fragments and chimeras, may be prepared using methods known to those skilled in the art.

4. **Applications of the Nucleic Acid Molecules, OB-BPL Related Proteins, and Antibodies of the Invention**

The nucleic acid molecules, OB-BPL Related Proteins, and antibodies of the invention may be used in the prognostic and diagnostic evaluation of cancer or disorders of the hematopoietic system, and the identification of subjects with a predisposition to cancer or hematopoietic disorders (Section 4.1.1 and 4.1.2). Methods for detecting nucleic acid molecules and OB-BPL Related Proteins of the invention, can be used to monitor cancer or hematopoietic disorders by detecting OB-BPL Related Proteins and nucleic acid molecules encoding OB-BPL Related Proteins. It would also be apparent to one skilled in the art that the methods described herein may be used to study the developmental expression of OB-BPL Related Proteins and, accordingly, will provide further insight into the role of OB-BPL Related Proteins. The applications of the present invention also include methods for the identification of compounds that modulate the biological activity of *OB-BPL* or OB-BPL Related Proteins (Section 4.2). The compounds, antibodies etc. may be used for the treatment of cancer or hematopoietic disorders (Section 4.3).

4.1 **Diagnostic Methods**

A variety of methods can be employed for the diagnostic and prognostic evaluation of cancer or disorders of the hematopoietic system (e.g. leukemias), and the identification of subjects with a predisposition to cancer or hematopoietic disorders. Such methods may, for example, utilize nucleic acid molecules of the invention, and fragments thereof, and antibodies directed against OB-BPL Related Proteins, including peptide fragments. In particular, the nucleic acids and antibodies may be used, for example, for: (1) the detection of the presence of *OB-BPL* mutations, or the detection of either over- or under-expression of *OB-BPL* mRNA relative to a non-disorder state or the qualitative or quantitative detection of alternatively spliced forms of *OB-BPL* transcripts which may correlate with certain conditions

or susceptibility toward such conditions; and (2) the detection of either an over- or an under-abundance of OB-BPL Related Proteins relative to a non- disorder state or the presence of a modified (e.g., less than full length) OB-BPL Protein which correlates with a disorder state, or a progression toward a disorder state.

The methods described herein may be performed by utilizing pre-packaged diagnostic kits comprising at least one specific *OB-BPL* nucleic acid or antibody described herein, which may be conveniently used, e.g., in clinical settings, to screen and diagnose patients and to screen and identify those individuals exhibiting a predisposition to developing a disorder.

Nucleic acid-based detection techniques are described, below, in Section 4.1.1. Peptide detection techniques are described, below, in Section 4.1.2. The samples that may be analyzed using the methods of the invention include those which are known or suspected to express *OB-BPL* or contain OB-BPL Related Proteins. The samples may be derived from a patient or a cell culture, and include but are not limited to biological fluids, tissue extracts, freshly harvested cells, and lysates of cells which have been incubated in cell cultures.

Oligonucleotides or longer fragments derived from any of the nucleic acid molecules of the invention may be used as targets in a microarray. The microarray can be used to simultaneously monitor the expression levels of large numbers of genes and to identify genetic variants, mutations, and polymorphisms. The information from the microarray may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

The preparation, use, and analysis of microarrays are well known to a person skilled in the art. (See, for example, Brennan, T. M. et al. (1995) U.S. Pat. No. 5,474,796; Schena, et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995), PCT Application WO95/251116; Shalon, D. et al. (I 995) PCT application WO95/35505; Heller, R. A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M. J. et al. (1997) U.S. Pat. No. 5,605,662.)

4.1.1 Methods for Detecting Nucleic Acid Molecules of the Invention

The nucleic acid molecules of the invention allow those skilled in the art to construct nucleotide probes for use in the detection of nucleic acid sequences of the invention in samples. Suitable probes include nucleic acid molecules based on nucleic acid sequences encoding at least 5 sequential amino acids from regions of the OB-BPL Protein, preferably they comprise 15 to 30 nucleotides. A nucleotide probe may be labeled with a detectable substance such as a radioactive label which provides for an adequate signal and has sufficient half-life such as ^{32}P, ^{3}H, ^{14}C or the like. Other detectable substances which may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and luminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization. Labeled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.). The nucleic acid probes may be used to detect genes, preferably in human cells, that encode OB-BPL Related Proteins. The nucleotide

probes may also be useful in the diagnosis of disorders of the hematopoietic system or cancer; in monitoring the progression of such disorders; or monitoring a therapeutic treatment.

The probe may be used in hybridization techniques to detect genes that encode OB-BPL Related Proteins. The technique generally involves contacting and incubating nucleic acids (e.g. recombinant DNA molecules, cloned genes) obtained from a sample from a patient or other cellular source with a probe of the present invention under conditions favorable for the specific annealing of the probes to complementary sequences in the nucleic acids. After incubation, the non-annealed nucleic acids are removed, and the presence of nucleic acids that have hybridized to the probe if any are detected.

The detection of nucleic acid molecules of the invention may involve the amplification of specific gene sequences using an amplification method such as PCR, followed by the analysis of the amplified molecules using techniques known to those skilled in the art. Suitable primers can be routinely designed by one of skill in the art.

Genomic DNA may be used in hybridization or amplification assays of biological samples to detect abnormalities involving *ob-bpl* structure, including point mutations, insertions, deletions, and chromosomal rearrangements. For example, direct sequencing, single stranded conformational polymorphism analyses, heteroduplex analysis, denaturing gradient gel electrophoresis, chemical mismatch cleavage, and oligonucleotide hybridization may be utilized.

Genotyping techniques known to one skilled in the art can be used to type polymorphisms that are in close proximity to the mutations an OB-BPL gene. The polymorphisms may be used to identify individuals in families that are likely to carry mutations. If a polymorphism exhibits linkage disequalibrium with mutations in an OB-BPL gene, it can also be used to screen for individuals in the general population likely to carry mutations. Polymorphisms which may be used include restriction fragment length polymorphisms (RFLPs), single-base polymorphisms, and simple sequence repeat polymorphisms (SSLPs).

A probe of the invention may be used to directly identify RFLPs. A probe or primer of the invention can additionally be used to isolate genomic clones such as YACs, BACs, PACs, cosmids, phage or plasmids. The DNA in the clones can be screened for SSLPs using hybridization or sequencing procedures.

Hybridization and amplification techniques described herein may be used to assay qualitative and quantitative aspects of *OB-BPL* expression. For example, RNA may be isolated from a cell type or tissue known to express *OB-BPL* and tested utilizing the hybridization (e.g. standard Northern analyses) or PCR techniques referred to herein. The techniques may be used to detect differences in transcript size which may be due to normal or abnormal alternative splicing. The techniques may be used to detect quantitative differences between levels of full length and/or alternatively splice transcripts detected in normal individuals relative to those individuals exhibiting symptoms of a hematopoietic disorder or other disease conditions.

The primers and probes may be used in the above described methods *in situ* i.e directly on tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections.

4.1.2 **Methods for Detecting OB-BPL Related Proteins**

Antibodies specifically reactive with an OB-BPL Related Protein, or derivatives, such as enzyme conjugates or labeled derivatives, may be used to detect OB-BPL Related Proteins in various samples (e.g. biological materials). They may be used as diagnostic or prognostic reagents and they may be used to detect abnormalities in the level of OB-BPL Related Proteins expression, or abnormalities in the structure, and/or temporal, tissue, cellular, or subcellular location of an OB-BPL Related Protein. Antibodies may also be used to screen potentially therapeutic compounds *in vitro* to determine their effects on disorders of the hematopoietic system, and other conditions. *In vitro* immunoassays may also be used to assess or monitor the efficacy of particular therapies. The antibodies of the invention may also be used *in vitro* to determine the level of *OB-BPL* expression in cells genetically engineered to produce an OB-BPL Related Protein.

The antibodies may be used in any known immunoassays which rely on the binding interaction between an antigenic determinant of an OB-BPL Related Protein and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, and histochemical tests. The antibodies may be used to detect and quantify OB-BPL Related Proteins in a sample in order to determine its role in particular cellular events or pathological states, and to diagnose and treat such pathological states.

In particular, the antibodies of the invention may be used in immuno-histochemical analyses, for example, at the cellular and sub-subcellular level, to detect an OB-BPL Related Protein, to localize it to particular cells and tissues, and to specific subcellular locations, and to quantitate the level of expression.

Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect an OB-BPL Related Protein. Generally, an antibody of the invention may be labeled with a detectable substance and an OB-BPL Related Protein may be localised in tissues and cells based upon the presence of the detectable substance. Examples of detectable substances include, but are not limited to, the following: radioisotopes (e.g., ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), luminescent labels such as luminol; enzymatic labels (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase), biotinyl groups (which can be detected by marked avidin e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods), predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached via spacer arms of various lengths to reduce potential steric hindrance. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualised by electron microscopy.

The antibody or sample may be immobilized on a carrier or solid support which is capable of immobilizing cells, antibodies etc. For example, the carrier or support may be nitrocellulose, or glass, polyacrylamides, gabbros, and magnetite. The support material may have any possible configuration including spherical (e.g. bead), cylindrical (e.g. inside surface of a test tube or well, or the external surface of a rod), or flat (e.g. sheet, test strip). Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against OB-BPL Related Protein. By way of example, if the antibody having specificity

against an OB-BPL Related Protein is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labeled with a detectable substance as described herein.

Where a radioactive label is used as a detectable substance, an OB-BPL Related Protein may be localized by radioautography. The results of radioautography may be quantitated by determining the density of particles in the radioautographs by various optical methods, or by counting the grains.

4.2 Methods for Identifying or Evaluating Substances/Compounds

The methods described herein are designed to identify substances that modulate the biological activity of an OB-BPL Related Protein including substances that bind to OB-BPL Related Proteins, or bind to other proteins that interact with an OB-BPL Related Protein, to compounds that interfere with, or enhance the interaction of an OB-BPL Related Protein and substances that bind to the OB-BPL Related Protein or other proteins that interact with an OB-BPL Related Protein. Methods are also utilized that identify compounds that bind to *OB-BPL* regulatory sequences.

The substances and compounds identified using the methods of the invention include but are not limited to peptides such as soluble peptides including Ig-tailed fusion peptides, members of random peptide libraries and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, phosphopeptides (including members of random or partially degenerate, directed phosphopeptide libraries), antibodies [e.g. polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, single chain antibodies, fragments, (e.g. Fab, $F(ab)_2$, and Fab expression library fragments, and epitope-binding fragments thereof)], and small organic or inorganic molecules. The substance or compound may be an endogenous physiological compound or it may be a natural or synthetic compound.

Substances which modulate an OB-BPL Related Protein can be identified based on their ability to bind to an OB-BPL Related Protein. Therefore, the invention also provides methods for identifying substances which bind to an OB-BPL Related Protein. Substances identified using the methods of the invention may be isolated, cloned and sequenced using conventional techniques. A substance that associates with a polypeptide of the invention may be an agonist or antagonist of the biological or immunological activity of a polypeptide of the invention.

The term "agonist", refers to a molecule that increases the amount of, or prolongs the duration of, the activity of the polypeptide. The term "antagonist" refers to a molecule which decreases the biological or immunological activity of the polypeptide. Agonists and antagonists may include proteins, nucleic acids, carbohydrates, or any other molecules that associate with a polypeptide of the invention.

Substances which can bind with an OB-BPL Related Protein may be identified by reacting an OB-BPL Related Protein with a test substance which potentially binds to an OB-BPL Related Protein, under conditions which permit the formation of substance-OB-BPL Related Protein complexes and removing and/or detecting the complexes. The complexes can be detected by assaying for substance-OB-BPL Related Protein complexes, for free substance, or for non-complexed OB-BPL Related Protein. Conditions which permit the formation of substance-OB-BPL Related Protein complexes may be selected having regard to factors such as the nature and amounts of the substance and the protein.

The substance-protein complex, free substance or non-complexed proteins may be isolated by

conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combinations thereof. To facilitate the assay of the components, antibody against OB-BPL Related Protein or the substance, or labeled OB-BPL Related Protein, or a labeled substance may be utilized. The antibodies, proteins, or substances may be labeled with a detectable substance as described above.

AN OB-BPL Related Protein, or the substance used in the method of the invention may be insolubilized. For example, an OB-BPL Related Protein, or substance may be bound to a suitable carrier such as agarose, cellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose polystyrene, filter paper, ion-exchange resin, plastic film, plastic tube, glass beads, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. The carrier may be in the shape of, for example, a tube, test plate, beads, disc, sphere etc. The insolubilized protein or substance may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling.

The invention also contemplates a method for evaluating a compound for its ability to modulate the biological activity of an OB-BPL Related Protein of the invention, by assaying for an agonist or antagonist (i.e. enhancer or inhibitor) of the binding of an OB-BPL Related Protein with a substance which binds with an OB-BPL Related Protein. The basic method for evaluating if a compound is an agonist or antagonist of the binding of an OB-BPL Related Protein and a substance that binds to the protein, is to prepare a reaction mixture containing the OB-BPL Related Protein and the substance under conditions which permit the formation of substance-OB-BPL Related Protein complexes, in the presence of a test compound. The test compound may be initially added to the mixture, or may be added subsequent to the addition of the OB-BPL Related Protein and substance. Control reaction mixtures without the test compound or with a placebo are also prepared. The formation of complexes is detected and the formation of complexes in the control reaction but not in the reaction mixture indicates that the test compound interferes with the interaction of the OB-BPL Related Protein and substance. The reactions may be carried out in the liquid phase or the OB-BPL Related Protein, substance, or test compound may be immobilized as described herein. The ability of a compound to modulate the biological activity of an OB-BPL Related Protein of the invention may be tested by determining the biological effects on cells.

It will be understood that the agonists and antagonists i.e. inhibitors and enhancers that can be assayed using the methods of the invention may act on one or more of the binding sites on the protein or substance including agonist binding sites, competitive antagonist binding sites, non-competitive antagonist binding sites or allosteric sites.

The invention also makes it possible to screen for antagonists that inhibit the effects of an agonist of the interaction of OB-BPL Related Protein with a substance which is capable of binding to the OB-BPL Related Protein. Thus, the invention may be used to assay for a compound that competes for the same binding site of an OB-BPL Related Protein.

The invention also contemplates methods for identifying compounds that bind to proteins that interact with an OB-BPL Related Protein. Protein-protein interactions may be identified using conventional

methods such as co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns. Methods may also be employed that result in the simultaneous identification of genes which encode proteins interacting with an OB-BPL Related Protein. These methods include probing expression libraries with labeled OB-BPL Related Protein.

Two-hybrid systems may also be used to detect protein interactions *in vivo*. Generally, plasmids are constructed that encode two hybrid proteins. A first hybrid protein consists of the DNA-binding domain of a transcription activator protein fused to an OB-BPL Related Protein, and the second hybrid protein consists of the transcription activator protein's activator domain fused to an unknown protein encoded by a cDNA which has been recombined into the plasmid as part of a cDNA library. The plasmids are transformed into a strain of yeast (e.g. *S. cerevisiae*) that contains a reporter gene (e.g. lacZ, luciferase, alkaline phosphatase, horseradish peroxidase) whose regulatory region contains the transcription activator's binding site. The hybrid proteins alone cannot activate the transcription of the reporter gene. However, interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

It will be appreciated that fusion proteins may be used in the above-described methods. In particular, OB-BPL Related Proteins fused to a glutathione-S-transferase may be used in the methods.

The reagents suitable for applying the methods of the invention to evaluate compounds that modulate an OB-BPL Related Protein may be packaged into convenient kits providing the necessary materials packaged into suitable containers. The kits may also include suitable supports useful in performing the methods of the invention.

4.3 Compositions and Treatments

The proteins of the invention, substances or compounds identified by the methods described herein, antibodies, and antisense nucleic acid molecules of the invention may be used for modulating the biological activity of an OB-BPL Related Protein, and they may be used in the treatment of conditions such as cancer and disorders of the hematopoietic system, in particular leukemias.

Hematopoietic disorders include but are not limited to myeloproliferative or other proliferative disorders of blood forming organs such as thromocythemias, polycythemias, and leukemias (acute myelogenous leukemia, chronic myelogenous leukemia). The proteins, substances, compounds, antibodies, and antisense nucleic acid molecules of the invention may be used in conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment, or chemotherapy. They may also be used to treat hematopoietic disorders associated with viral or bacterial infections.

Accordingly, the substances, antibodies, peptides, and compounds may be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the active substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The active substances may be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of a pharmaceutical composition of the present invention

is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the substance from the action of enzymes, acids and other natural conditions that may inactivate the substance.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the active substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

Vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used to deliver nucleic acid molecules to a targeted organ, tissue, or cell population. Methods well known to those skilled in the art may be used to construct recombinant vectors which will express antisense nucleic acid molecules of the invention. (See, for example, the techniques described in Sambrook et al (supra) and Ausubel et al (supra)).

The nucleic acid molecules comprising full length cDNA sequences and/or their regulatory elements enable a skilled artisan to use sequences encoding a protein of the invention as an investigative tool in sense (Youssoufian H and H F Lodish 1993 Mol Cell Biol 13:98-104) or antisense (Eguchi et al (1991) Annu Rev Biochem 60:631-652) regulation of gene function. Such technology is well known in the art, and sense or antisense oligomers, or larger fragments, can be designed from various locations along the coding or control regions.

Genes encoding a protein of the invention can be turned off by transfecting a cell or tissue with vectors which express high levels of a desired OB-BPL-encoding fragment. Such constructs can inundate cells with untranslatable sense or antisense sequences. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies are disabled by endogenous nucleases.

Modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA or PNA, to the regulatory regions of a gene encoding a protein of the invention, ie, the promoters, enhancers, and introns. Preferably, oligonucleotides are derived from the transcription initiation site, eg,

between -10 and +10 regions of the leader sequence. The antisense molecules may also be designed so that they block translation of mRNA by preventing the transcript from binding to ribosomes. Inhibition may also be achieved using "triple helix" base-pairing methodology. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Therapeutic advances using triplex DNA were reviewed by Gee J E et al (In: Huber B E and B I Carr (1994) Molecular and Immunologic Approaches, Futura Publishing Co, Mt Kisco N.Y.).

Ribozymes are enzymatic RNA molecules that catalyze the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. The invention therefore contemplates engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding a protein of the invention.

Specific ribozyme cleavage sites within any potential RNA target may initially be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once the sites are identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be determined by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Methods for introducing vectors into cells or tissues include those methods discussed herein and which are suitable for *in vivo*, *in vitro* and *ex vivo* therapy. For *ex vivo* therapy, vectors may be introduced into stem cells obtained from a patient and clonally propagated for autologous transplant into the same patient (See U.S. Pat. Nos. 5,399,493 and 5,437,994). Delivery by transfection and by liposome are well known in the art.

The nucleic acid molecules disclosed herein may also be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including but not limited to such properties as the triplet genetic code and specific base pair interactions.

The invention also provides methods for studying the function of a polypeptide of the invention. Cells, tissues, and non-human animals lacking in expression or partially lacking in expression of a nucleic acid molecule or gene of the invention may be developed using recombinant expression vectors of the invention having specific deletion or insertion mutations in the gene. A recombinant expression vector may be used to inactivate or alter the endogenous gene by homologous recombination, and thereby create a deficient cell, tissue, or animal.

Null alleles may be generated in cells, such as embryonic stem cells by deletion mutation. A recombinant gene may also be engineered to contain an insertion mutation that inactivates the gene. Such a construct may then be introduced into a cell, such as an embryonic stem cell, by a technique such as transfection, electroporation, injection etc. Cells lacking an intact gene may then be identified, for example by Southern blotting, Northern Blotting, or by assaying for expression of the encoded polypeptide using

conditions used for the PCR reaction were identical to those discussed previously, with electrophoresis of the PCR product on a 2% agarose gel, gel extraction, and automated sequencing as before.

Following final characterization of the genomic structure of this novel siglec, the putative protein product was aligned with the protein sequences of the other siglec family members using the ClustalX multiple sequence alignment tool. Further, phylogenetic analysis was performed using ClustalX in combination with TreeView (Page 1996).

Sequence analysis tools, available through the internet, were also utilized to detect the presence of possible sites of post-translational modification on the putative protein. The analysis programs PROSITE motif search (http://www.expasy.ch/prosite/) (Bairoch *et al.*, 1997), and NetOGlyc 2.0 (Hansen *et al.*, 1995; Hansen *et al.*, 1998) were used to detect N- and O-glycosylation, as well as the presence of kinase phosphorylation motifs. Further, the putative protein was assessed for the presence of a possible signal peptide, using SignalP v1.1 (http://www.cbs.dtu.uk/) (Nielsen *et al.*, 1997). For the prediction of transmembrane domains, two independent algorithms were used, TMpred (http://www.ch.embnet.org/software/TMPRED_form.html) and DAS (http://www.biokemi.su.se/~server/). In addition, the hydropathic profile of this novel siglec was determined, using the Kyte-Doolittle method (http://bioinformatics.weizmann.ac.il/hydroph/plot_hydroph.html).

Mapping and Chromosomal Localization of a Novel Siglec

As mentioned previously, the contig on which the novel siglec gene was identified was obtained from the LLNL. EcoRI restriction maps were obtained from LLNL, and also generated using the Webcutter restriction analysis tool (http://www.firstmarker.com/cutter/cutter2.html), for both this contig, as well as the adjacent more centromeric contigs, containing the recently identified kallikrein gene family (Diamandis *et al.*, 1999; Yousef *et al.*, 1999a). Overlapping restriction fragments were identified and used to order the contigs and determine the distance between KLK-L6, the most telomeric member of the kallikrein gene family, and this novel siglec.

Tissue Expression

Total RNA from 28 normal human tissues was obtained (Clontech, Palo Alto, CA, USA), and reverse transcription was performed using SuperScript II™, according to the manufacturer's instructions (Gibco BRL, Gaithersburg, MD, USA). PCR was then performed using primers F2 (CGTGGGAGATACGGGCATAG - SEQ.ID.NO.8) and R2 (AAAAGGGAGGGCACAGTGTG - SEQ.ID.NO. 9), using the same PCR conditions described previously. PCR for actin was also performed as describe elsewhere (Yousef *et al.*, 1999b), as a control for cDNA quality.

RESULTS

Identification of a Novel Siglec on 19q13.4

Computer analysis of the approximately 130 Kb contig predicted a putative new gene consisting of six exons. Five of these were predicted by at least three programs, with only one exon being predicted by two of the four programs (Table 1). Homology search for the putative new gene against the human EST database revealed the presence of one unique EST (GenBank accession # AA936059) which showed 98% identity to the sixth predicted exon.

The entire insert of this EST was sequenced, followed by alignment of this nucleotide sequence with the genomic sequence of the putative gene, using the "BLAST 2 sequences" program. This revealed the presence of an additional area, between predicted exons 5 and 6, with 98% identity to the EST. This suggested that there was an additional exon in this area which was not detected by the prediction algorithms used.

Characterization of the Genomic Structure of the Novel Siglec Gene and its Protein Product

With the aid of unique primers, designed as discussed in the experimental section, RT-PCR was performed on bone marrow cDNA and two additional products were isolated, both encompassing multiple predicted exons. Upon sequencing of these PCR products, the presence of all six predicted exons, as well as the newly identified exon, found from the EST sequence were confirmed. With both cDNA and genomic sequence at hand, the genomic organization of this new gene was determined (Figure 1). The gene encoding this novel siglec encompasses a genomic area of 5,421 bp. It is composed of seven exons, with six intervening introns. The lengths of the exons are 509, 279, 48, 267, 91, 97, and 417 bp, respectively. All the intron/exon splice sites and their flanking sequences are closely related to the consensus splice sites (-mGTAAGT...CAGm-, where m is any base) (Iida 1990).

The proposed protein coding region of the novel siglec gene consists of 1,392 nucleotides, producing a 463 amino acid protein, with a predicted molecular mass of 50.1 kDa, excluding any post-translational modifications. The translation initiation codon (ATG) at position 1171 of the first exon (according to the numbering of SEQ. ID. NO. 1 and GenBank Accession No. AF135027), was chosen because: 1) the flanking region surrounding that codon closely matches the Kozak consensus sequence for translational initiation, particularly at position –3 (a purine), which appears to be the most highly conserved (Kozak 1991); 2) using this initiation codon, the proposed protein contains an N-terminal signal sequence which shows a high degree of homology to other similar proteins (see below). The 3' terminus of the novel siglec gene was verified by the presence of a poly dA tail present in the EST sequence. Further, it is evident from Figure 1 that this gene possesses a 5' untranslated region of at least 88 nucleotides, as well as a 3' untranslated region of 228 nucleotides.

Examination of the hydrophobicity profile of the novel siglec protein revealed two regions with long stretches of hydrophobic residues. The first of these occurs at the N-terminus, suggesting the presence of a signal peptide (Figure 2). This is consistent with findings from a signal sequence prediction program (Nielsen *et al.*, 1997), which predicts a 17 amino acid residue signal sequence. The second region occurs between residues 349 and 370, suggestive of a transmembrane domain, and is consistent with results from transmembrane region prediction programs. Based on this information, the protein product of this novel gene is likely a type I transmembrane protein, after cleavage of the 17 residue signal sequence.

Through the use of sequence analysis tools, the various putative post-translational modification sites were identified (Table 2). There are numerous potential sites in this novel siglec where there could be either O- or N-glycosylation. Furthermore, several possible sites of phosphorylation have been identified for cAMP-dependent protein kinase, protein kinase C, and casein kinase 2.

Mapping and Chromosomal Localization of a Novel Siglec

phosphorylation of the ITIM-like motif in CD22, the phosphatase SHP-1 is recruited, suggesting a possible function of this siglec as a B cell receptor-associated negative co-receptor (Vivier and Daeron 1997). The second cytoplasmic motif has been identified in SLAM and several SLAM-like proteins, a family of immunoregulatory molecules of the IgSF, and is responsible for the binding of a new SH2-containing molecule, SAP (Coffey *et al.*, 1998; Sayos *et al.*, 1998). The binding of SAP was shown to inhibit the binding of SHP-2 to its respective binding site on these SLAM proteins. The presence of such a motif in the novel siglec, and other siglecs, suggests that there may be a similar regulatory mechanism present in the cytoplasmic domains of siglecs, with SAP inhibiting the binding of SHP-1 and SHP-2 to the ITIM-like motif.

The regulation of SHP-1 and SHP-2 binding to ITIM motifs, and thus their activation, very likely affects downstream tyrosine-kinase dependent pathways by regulating the phosphorylation state of components in these pathways. Thus, the siglec family of ITIM and SLAM-bearing receptors probably play a role in controlling the activation of a number of cell types. By extension, it is possible that these siglecs may be involved in the regulation of tumour growth. CD33 has already been identified as an important marker for the diagnosis of acute myelogenous leukemia (AML), particularly for the undifferentiated form, and serves to distinguish AML from lymphoid leukemias (Bernstein *et al.*, 1992; Dinndorf *et al.*, 1986; Griffin *et al.*, 1984). Recently, Kossman et. al. and Sievers et. al. have reported the use of anti-CD33 monoclonal antibodies in phase I studies for the treatment of AML, and have shown selective ablation of malignant hematopoiesis (Kossman *et al.*, 1999; Sievers *et al.*, 1999). The newly identified member of the siglec family may have utility as a target for immunological antineoplastic therapy.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated to those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. All modifications coming within the scope of the following claims are claimed.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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Table 1: Genomic organization of a novel siglec.

Exon No.	Coding Region[1]		No. of base pairs	EST Match[2]	Intron Phase	Exon Predicted[3]
	From (bp)	To (bp)				
1	1083	1591	509	-	I	B,C
2	1793	2071	279	-	I	A,B,C,D
3	2277	2324	48	-	I	A,B,D
4	3226	3492	267	-	I	A,B,C
5	4145	4235	91	-	0	A,B,C,D
6	4610	4706	97	+	0	-
7	6087	6503	417	+	-	A,B,C

1. The coding region shown includes the 5' untranslated region in exon 1, and the 3' untranslated region in exon 7. Numbers refer to GenBank accession no. AF135027.
2. EST; GenBank accession no. AA936059
3. The exon prediction programs are as follows: A) GeneBuilder (gene prediciton); B) GeneBuilder (exon prediction); C) Grail 2; D) GENEID-3.

Table 2: Putative post-translational modification sites in the novel siglec.

Modification[1]	Residue	Position[2]
O-glycosylation	Thr	76, 192, 193
	Ser	184, 186, 195
N-glycosylation	Asn	101, 138, 161, 225, 231, 238, 256, 334
cAMP-dependent Protein Kinase phosphorylation	Ser/Thr	374
Protein Kinase C phosphorylation	Ser/Thr	372, 377, 421
Casein Kinase 2 phosphorylation	Ser/Thr	387, 412, 425, 452

1. The proposed O-glycosylation sites were determined through NetOGlyc 2.0 (Hansen *et al.*, 1998). The remainder of the post-translational modifications were predicted by PROSITE (Hansen *et al.*, 1995).
2. The residue numbering is according to the numbering of the novel siglec, as shown in Figure 4.

Table 3: Overall homology of this novel siglec with other known siglecs.

Siglec Family Member[1]	Homology to the Novel Siglec[2]	
	% identity	% similarity
Siglec-7 (p75/AIRM1) (AF170485)	75	80
Siglec-5 (OB-BP2) (U71383)	52	65
CD33 (M23197)	52	64
Siglec-6 (OB-BP1) (U71382)	49	60
Sialoadhesin (Z36293)	27	43
CD22 (X52785)	26	42
Myelin associated glycoprotein (MAG) (M29273)	25	42

1. GenBank accession numbers for each of the siglec family members is also shown, in brackets.
2. Homology was determined using the BLASTP algorithm (Altschul *et al.*, 1997).

Table 4: Ig-like domain homology between the novel siglec and other siglec family members[1]

	Homologous Protein	Domain	% identity	% similarity
Novel Siglec Ig 1 (V-set)	Siglec-7 (p75/AIRM1)	1	75	78
	CD33	1	61	71
	Siglec-5 (OB-BP2)	1	54	67
	Siglec-6 (OB-BP1)	1	54	62
	MAG	1	32	48
	Sialoadhesin	1	29	48
	CD22	1	28	44
Novel Siglec Ig 2 (C2-set)	Siglec-7 (p75/AIRM1)	2	89	93
	CD33	2	63	75
	Siglec-6 (OB-BP1)	2	58	70
	Siglec-5 (OB-BP2)	2	58	71
	Sialoadhesin	2	30	46
		12	31	44
	MAG	2	25	46
	CD22	2	27	43
Novel Siglec Ig 3 (C2-set)	Siglec-7 (p75/AIRM1)	3	76	79
	Siglec-6 (OB-BP1)	3	52	67
	Siglec-5 (OB-BP2)	3	48	62
	Sialoadhesin	13	33	48
		7	31	42
		15	28	40
	MAG	3	27	49

1. GenBank accession numbers for the listed siglecs are the same as those shown in Table 3.

Table 5. Predicted exons of the unknown gene UG . The translated protein sequences of each exon (open reading frame) are shown

Exon No.	Putative coding region[1] From(bp)	To(bp)	No. of bases	Translated protein sequence	EST match[2]	Intron phase[3]	Stop codon[4]	Exon prediction program[5]
1	44,129	44,641	513	PPLSLEPAVPERRTLRNRRSLAALAPLTPDMLLLLLPLL WGRERAEGQTSKLLTMQSSVTVQEGLCVHVPCSFSYPS HGWIYPGPVVHGYWFREGANTDQDAPVATNNPARAV WEETRDRFHLLGDPHTKNCTLSIRDARRSDAGRYFFRM EKGSIKWNYKHH RLSVNVT	+	I	-	B,C
2	44,843	45,121	279	ALTHRPNILIPGTLESGCPQNLTCSVPWACEQGTPPMIS WIGTSVSPLDPSTTRSSVLTLIPQPQDHGTSLTCQVTFPG ASVTTNKTVHLNVS	+	I	-	A,B,C,D
3	45,327	45,374	48	YPPQNLTMTVFQGDGT	-	I	-	A,B,D
4	46,318	46,542	225	EGQSLRLVCAVDAVDSNPPARLSLSWRGLTLCPSQPSN PGVLELPWVHLRDAAEFTCRAQNPLGSQQVYLNVSLQ	+	I	-	A,B,C
5	47,195	47,283	186	SKATSGVTQGVVGGAGATALVFLSFCVIFV	+	0	-	A,B,C,D
6	49,136	49,554	186	GPLTEPWAEDSPPDQPPPASARSSVGEGELQYASLSFQ MVKPWDS RGQEATDTEYSEIKIHR	+	-	+	A,B,C

* All footnotes same as table 2.

1. Conventional numbering of exons in comparison to the five coding exons of PSA.
   Nucleotide numbers refer to the related contig (see text).
2. (+) = >95% homology with published human EST sequences.
3. Intron phase:0=the intron occurs between codons; I=the intron occurs after the first nucleotide of the codon;
   II=the intron occurs after the second nucleotide of the codon.
4. (+) denotes the exon containing the stop codon.
5. H=histidine, D=aspartic acid, S=serine. The aminoacids of the catalytic triad are bold and underlined.
   A = GeneBuilder (gene analysis), B = GeneBuilder (exon analysis), C = Grail 2, D = GENEID-3

We Claim:

1. An isolated OB-BPL nucleic acid molecule of at least 30 nucleotides which hybridizes to SEQ ID NO. 1, or the complement of SEQ ID NO. 1, under stringent hybridization conditions.
2. An isolated nucleic acid molecule which comprises:
   (i) a nucleic acid sequence encoding a polypeptide having substantial sequence identity with the amino acid sequence shown in Table 5 or SEQ. ID. NO.2 or SEQ. ID. NO 3.;
   (ii) nucleic acid sequences complementary to (i);
   (iii) a degenerate form of a nucleic acid sequence of (i);
   (iv) a nucleic acid sequence comprising at least 18 nucleotides and capable of hybridizing to a nucleic acid sequence in (i), (ii), or (iii);
   (v) a nucleic acid sequence encoding a truncation, an analog, an allelic or species variation of a polypeptide comprising the amino acid sequence shown in Table 5 or SEQ. ID. NO.2, or SEQ. ID. NO 3; or
   (vi) a fragment, or allelic or species variation of (i), (ii) or (iii).
3. An isolated nucleic acid molecule which comprises:
   (a) a nucleic acid sequence having substantial sequence identity or sequence similarity with a nucleic acid sequence of SEQ. ID. NO. 1;
   (b) nucleic acid sequences complementary to (i), preferably complementary to the full nucleic acid sequence of SEQ. ID. NO. 1;
   (c) nucleic acid sequences differing from any of the nucleic acid sequences of (i) or (ii) in codon sequences due to the degeneracy of the genetic code; or
   (d) a fragment, or allelic or species variation of (i), (ii) or (iii).
4. An isolated nucleic acid molecule which encodes a protein which binds an antibody of a OB-BPL polypeptide.
5. A regulatory sequence of an isolated nucleic acid molecule as claimed in any of the preceding claims fused to a nucleic acid which encodes a heterologous protein.
6. A vector comprising a nucleic acid molecule of any of the preceding claims.
7. A host cell comprising a nucleic acid molecule of any of the preceding claims.
8. An isolated OB-BPL protein comprising an amino acid sequence of SEQ. ID. NO. 2 or 3.
9. An isolated protein having at least 65% amino acid sequence identity to an amino acid sequence of SEQ. ID. NO. 2 or 3.
10. A method for preparing a protein as claimed in claim 9 comprising:
    (a) transferring a vector as claimed in claim 7 into a host cell;

(b) selecting transformed host cells from untransformed host cells;

(c) culturing a selected transformed host cell under conditions which allow expression of the protein; and

(d) isolating the protein.

11. A protein prepared in accordance with the method of claim 11.

12. An antibody having specificity against an epitope of a polypeptide as claimed in claim 9.

13. An antibody as claimed in claim 13 labeled with a detectable substance and used to detect the polypeptide in biological samples, tissues, and cells.

14. A probe comprising a sequence encoding a protein as claimed in claim 9, or a part thereof.

15. A method of diagnosing and monitoring conditions mediated by a protein as claimed in claim 9 by determining the presence of a nucleic acid molecule as claimed in any of the preceding claims or a polypeptide as claimed in any of the preceding claims.

16. A method as claimed in claim 16 wherein the condition is cancer or a disorder of the hematopoietic system.

17. A method for identifying a substance which associates with a protein as claimed in claim 9 comprising (a) reacting the protein with at least one substance which potentially can associate with the protein, under conditions which permit the association between the substance and protein, and (b) removing or detecting protein associated with the substance, wherein detection of associated protein and substance indicates the substance associates with the protein.

18. A method for evaluating a compound for its ability to modulate the biological activity of a protein as claimed in claim 9 comprising providing a known concentration of the protein with a substance which associates with the protein and a test compound under conditions which permit the formation of complexes between the substance and protein, and removing and/or detecting complexes.

19. A method for detecting a nucleic acid molecule encoding a protein comprising an amino acid sequence of SEQ. ID. NO. 2 or 3 in a biological sample comprising the steps of:

(a) hybridizing a nucleic acid molecule of claim 2 to nucleic acids of the biological sample, thereby forming a hybridization complex; and

(b) detecting the hybridization complex wherein the presence of the hybridization complex correlates with the presence of a nucleic acid molecule encoding the protein in the biological sample.

20. A method as claimed in claim 20 wherein nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.
21. A method for treating a condition mediated by a protein as claimed in claim 9 comprising administering an effective amount of an antibody as claimed in claim 13 or a substance or compound identified in accordance with a method claimed in claim 18 or 19.
22. A method as claimed in claim 22 wherein the condition is a disorder of the hematopoietic system.
23. A composition comprising one or more of a nucleic acid molecule or prtoein claimed in any of the preceding claims, or a substance or compound identified using a method as claimed in any of the preceding claims, and a pharmaceutically acceptable carrier, excipient or diluent.
24. Use of one or more of a nucleic acid molecule or protein claimed in any of the preceding claims, or a substance or compound identified using a method as claimed in any of the preceding claims in the preparation of a pharmaceutical composition for treating a condition mediated by a polypeptide as claimed in claim 9.
25. A transgenic non-human mammal which doe not express an OB-BPL protein as claimed in claim 9 resulting in an OB-BPL associated pathology.
26. A transgenic animal assay system which provides a model system for testing for an agent that reduces or inhibits an OB-BPLassociated pathology

(a) administering the agent to a transgenic non-human animal as claimed in claim 26; and

(b) determining whether said agent reduces or inhibits an OB-BPL associated pathology in the transgenic non-human animal relative to a transgenic non-human animal of step (a) which has not been administered the agent.

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(54) Title: SIALIC ACID–BINDING IG–LIKE LECTIN (SIGLEC) GENE; OB–BINDING PROTEIN LIKE (OB–BPL)

(57) Abstract

The invention relates to nucleic acid molecules, proteins encoded by such nucleic acid molecules; and use of the proteins and nucleic acid molecules.

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Figure 1

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 L   T   M   Q   S   S   V   T   V   Q   E   G   L   C   V   H   V   P   C   S   F   S
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 Y   P   S   H   G   W   I   Y   P   G   P   V   V   H   G   Y   W   F   R   E   G   A
AAT ACA GAC CAG GAT GCT CCA GTG GCC ACA AAC AAC CCA GCT CGG GCA GTG TGG GAG GAG ACT CGG
 N   T   D   Q   D   A   P   V   A   T   N   N   P   A   R   A   V   W   E   E   T   R
GAC CGA TTC CAC CTC CTT GGG GAC CCA CAT ACC AAG AAT TGC ACC CTG AGC ATC AGA GAT GCC AGA
 D   R   F   H   L   L   G   D   P   H   T   K   N   C   T   L   S   I   R   D   A   R
AGA AGT GAT GCG GGG AGA TAC TTC TTT CGT ATG GAG AAA GGA AGT ATA AAA TGG AAT TAT AAA CAT
 R   S   D   A   G   R   Y   F   F   R   M   E   K   G   S   I   K   W   N   Y   K   H
CAC CGG CTC TCT GTG AAT GTG ACA Ggtaa...INTRON1...cagCC TTG ACC CAC AGG CCC AAC ATC CTC
 H   R   L   S   V   N   V   T                         A   L   T   H   R   P   N   I   L
ATC CCA GGC ACC CTG GAG TCC GGC TGC CCC CAG AAT CTG ACC TGC TCT GTG CCC TGG GCC TGT GAG
 I   P   G   T   L   E   S   G   C   P   Q   N   L   T   C   S   V   P   W   A   C   E
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 Q   G   T   P   P   M   I   S   W   I   G   T   S   V   S   P   L   D   P   S   T   T
CGC TCC TCG GTG CTC ACC CTC ATC CCA CAG CCC CAG GAC CAT GGC ACC AGC CTC ACC TGT CAG GTG
 R   S   S   V   L   T   L   I   P   Q   P   Q   D   H   G   T   S   L   T   C   Q   V
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TGC CCC TCA CAG CCC TCA AAC CCG GGG GTG CTG GAG CTG CCT TGG GTG CAC CTG AGG GAT GCA GCT
 C   P   S   Q   P   S   N   P   G   V   L   E   L   P   W   V   H   L   R   D   A   A
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 E   F   T   C   R   A   Q   N   P   L   G   S   Q   Q   V   Y   L   N   V   S   L   Q
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                       S   K   A   T   S   G   V   T   Q   G   V   V   G   G   A   G   A
ACA GCC CTG GTC TTC CTG TCC TTC TGC GTC ATC TTC GTT GTgtaa..  INTRON5   ..cagA GTG AGG
 T   A   L   V   F   L   S   F   C   V   I   F   V   V                       V   R
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 S   C   R   K   K   S   A   R   P   A   A   G   V   G   D   T   G   I   E   D   A   N
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 E   D   S   P   P   D   Q   P   P   P   A   S   A   R   S   S   V   G   E   G   E   L
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 Q   Y   A   S   L   S   F   Q   M   V   K   P   W   D   S   R   G   Q   E   A   T   D
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 T   E   Y   S   E   I   K   I   H   R   *
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Figure 2

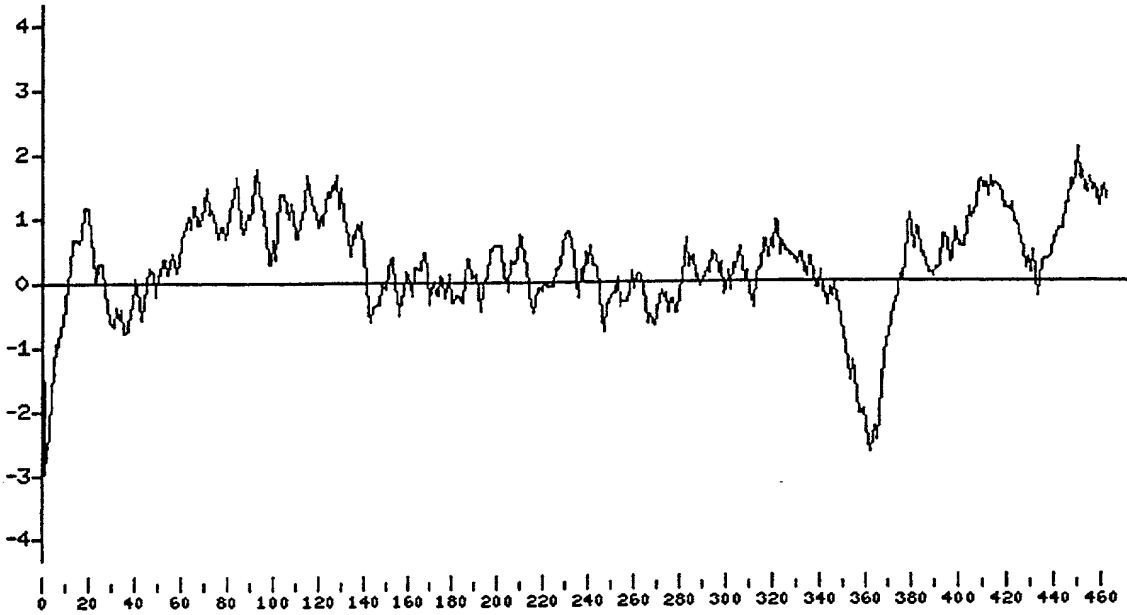


Figure 3

09/936278

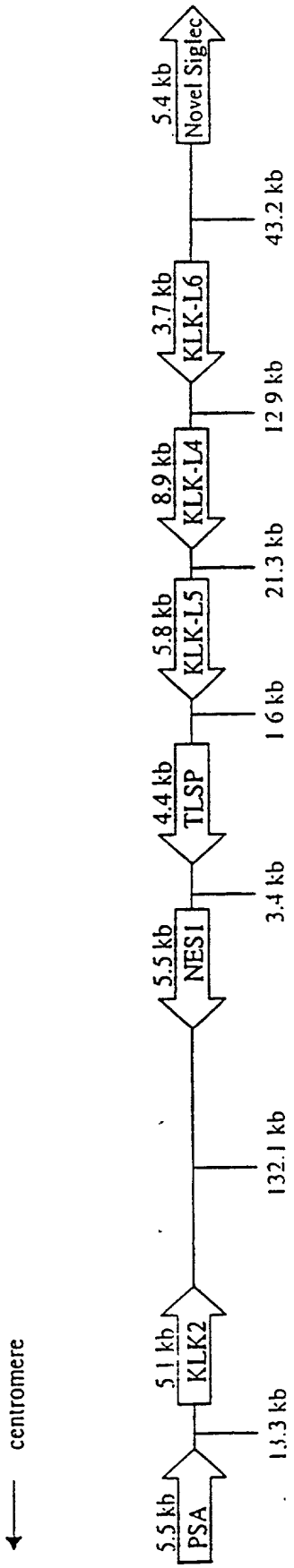


Figure 4

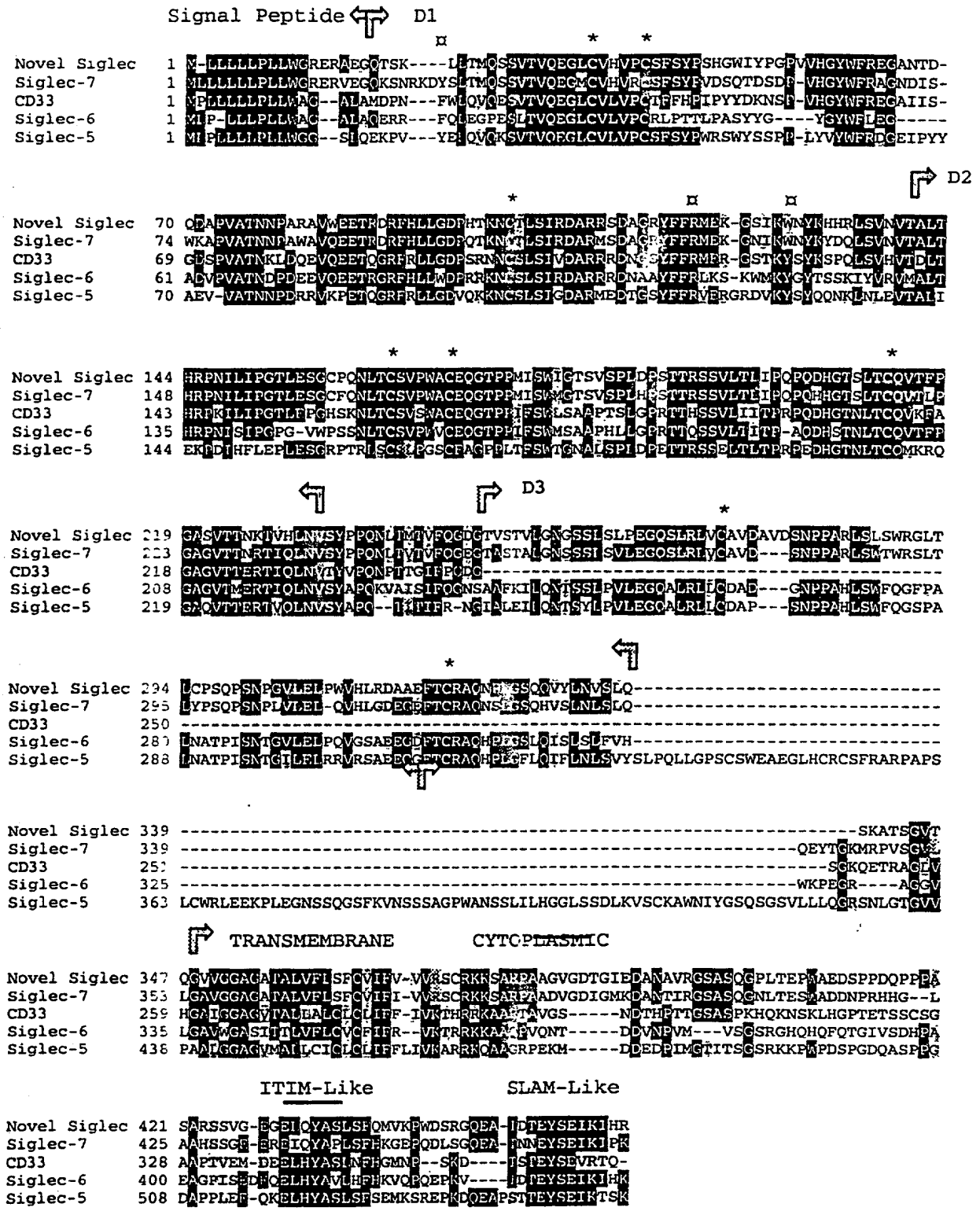
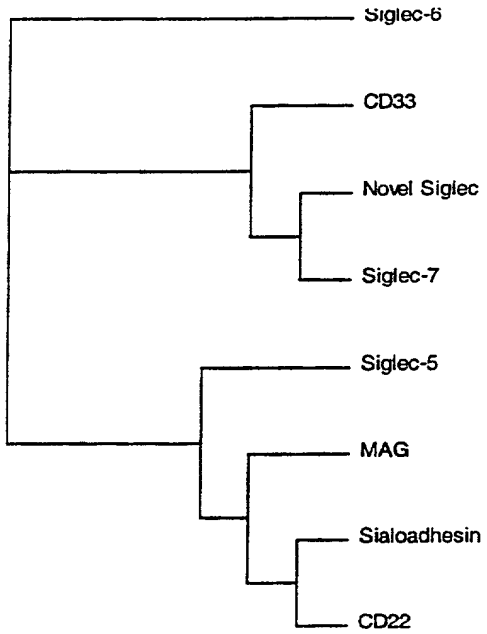
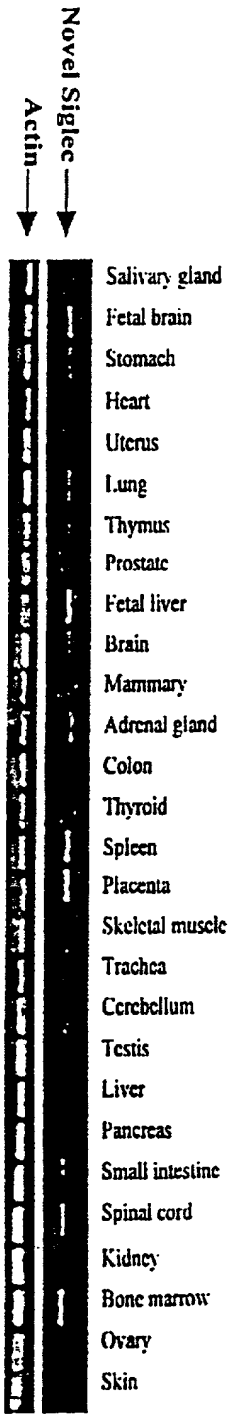


Figure 5



6/6

Figure 6



DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled SIALIC ACID-BINDING IG-LIKE LECTIN (SIGLEC) GENE; OB-BINDING PROTEIN LIKE (OB-BPL), the specification of which

(check one)
- ___ is attached hereto
- X was filed on September 10, 2001 as Application No. 09/936,278 and was amended on September 10, 2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed	Certified Copy Attached?	
(Number)	(Country)	(MM/DD/YYYY)	___	Yes	No

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date, MM/DD/YYYY)
60/144,919	July 21, 1999
60/127,386	April 1, 1999
60/124,260	March 11, 1999

I hereby appoint the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: STANLEY B. KITA, Registration No. 24,561; GEORGE A. SMITH, JR., Registration No. 24,442; MARY E. BAK, Registration No. 31,215, CATHY A. KODROFF, Registration Number 33,980, WILLIAM BAK, Registration Number 37,277, HENRY HANSEN, Registration No. 19,612, and TRACY U. PALOVICH, Registration No. 47,840

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Sequence Listing

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SEQ.ID.NO. 11

CD33

MPLLLLLPLLWAGALAMDPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNSPVHGYWFREG
AIISGDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKS
PQLSVHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIIT
PRPQDHGTNLTCQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGLVHGAIGGA
GVTALLALCLCLIFFIVKTHRRKAARTAVGSNDTHPTTGSASPKHQKNSKLHGPTETSSCSGAAPT
VEM-DEELHYASLNFHGMNPSKDTSTEYSEVRTQ

SEQ.ID.NO. 12

Siglec-6

MLP-LLLPLLWAGALAQERRFQLEGPESLTVQEGLCVLVPCRLPTTLPASYYGYGYWFLEG
ADVPVATNDPDEEVQEETRGRFHLLWDPRRKNCSLSIRDARRRDNAAYFFRLKSKWMKYGYTSS
KIYVRVMALTHRPNISIPGPGVWPSSNLTCSVPWVCEQGTPPIFSWMSAAPHLLGPRTTQSSVLTIT
PAQDHSTNLTCQVTFPGAGVTMERTIQLNVSYAPQKVAISIFQGNSAAFKILQNTSSLPVLEGQALR
LLCDADGNPPAHLSWFQGFPALNATPISNTGVLELPQVGSAEEGDFTCRAQHPLGSLQISLSLFVH
WKPEGRAGGVLGAVWGASITTLVFLCVCFIFRVKTRRKKAAQPVQNTDDVNPVMVSGSRGHQHQ
FQTGIVSDHPA EAGPISEDEQELHYAVLHFHKVQPQEPKVTDTEYSEIKIHK

SEQ.ID.NO. 13

Siglec-5

MLPLLLLPLLWGGSLQEKPVYELQVQKSVTVQEGLCVLVPCSFSYPWRSWYSSPPLYVY
WFRDGEIPYYAEVVATNNPDRRVKPETQGRFRLLGDVQKKNCSLSIGDARMEDTGSYF
RVERGRDVKYSYQQNKLNLEVTALIEKPDIHFLEPLESGRPTRLSCSLPGSCEAGPPLT
FSWTGNALSPLDPETTRSSELTLTPRPEDHGTNLTCQMKRQGAQVTTERTVQLNVSYAPQT
ITIFRNGIALEILQNTSYLPVLEGQALRLLCDAPSNPPAHLSWFQGSPALNATPISNTGILELRRVRSA
EEGGFTCRAQHPLGFLQIFLNLSVYSLPQLLGPSCSWEAEGLHCRCSFRARPAPSLCWRLEEKPLEG
NSSQGSFKVNSSSAGPWANSSLILHGGLSSDLKVSCKAWNIYGSQSGSVLLLQGRSNLGTGVVPAA
LGGAGVMALLCICLCLIFFLIVKARRKQAAGRPEKMDDEDPIMGTITSGSRKKPWPDSPGDQASPP
GDAPPLEE-QKELHYASLSFSEMKSREPKDQEAPSTTEYSEIKTSK